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The Effect on the Cyst Contents of *Heterodera schachtii* of the Cultivation of Maize on Potato Sick Land.

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Field observations made in 1935 on a potato crop growing on what was thought to be potato sick land in Bedfordshire seemed to indicate that eelworm damage due to *Heterodera schachtii* was less pronounced where maize had been the previous crop than in other cases. As particulars of the history of this piece of ground were not available, detailed observations were not made, but it was decided that it might be valuable to carry out experiments with maize to determine whether this plant had any effect on eelworm cysts. Other members of the natural order Gramineae have been shown by Triffitt to stimulate the larvae of *H. schachtii* to hatch from the cysts, and it was thought that maize might have a similar effect.

Preliminary hatching trials were first undertaken in which comparisons were made of the rates of hatching of larvae from cysts soaking in solutions containing excretions from potato roots and from maize roots, and in water alone. A first experiment showed a greater rate of hatching of larvae in maize root excretion than in water, but on repetition, approximately equal numbers hatched in the two liquids. A third trial gave similar results to the second. The cysts in potato root excretion produced many more larvae than in either of the other two liquids. The results of hatching experiments were therefore not significant.

Pot and field experiments were then made in order to compare the viable contents of cysts before and after the growth of maize.

Cyst Dissections.

The estimations of the viable contents of the cysts were made in very much the same way as that described by Triffitt (1934). Each cyst was dissected in a drop of water under the binocular microscope ; the contents were separated out with needles and the proportion of unhatched eggs was estimated. The cysts were classified as having the full complement of unhatched eggs, or as being three-quarters full, half-full, one-quarter full or empty. One hundred cysts from each sample were dissected,

and the percentage "fulness" or "viability" for the sample was calculated. The only difference in the method from that employed by Triffitt was that, instead of choosing cysts of equal size for dissection, the required number was removed from the sample taken for estimation by means of a moistened paint brush. In this way cysts of all sizes were picked up, and it was thought that a more general idea of the state of the cyst population was obtained.

Pot Experiments.

For the pot experiments soil was used from the locality where the field trials were to be carried out. The soil was known to be fairly heavily infested with *H. schachtii*, but no accurate cyst counts were made. Cysts were removed from a sample of the soil by the usual flotation method, and were kept for viability determinations. Six large plant pots were filled with the soil. One pot was sown with white maize and one with yellow maize, in order to compare the effect of the two varieties. Two pots were sown with a mixture of rough and smooth stalked meadow grasses (*Poa trivialis* and *P. pratensis*), and two with *Alopecurus pratensis*. The two grasses were useful for comparison with maize, since Triffitt had shown that the meadow grasses have a stimulatory effect on the larvae, while *A. pratensis* has no such effect. During the growing season the roots of the maize plants in each of the pots were carefully examined for eelworm cysts, but none could be found. At the end of the season the pots were turned out, the soil was thoroughly mixed and dried, and samples of cysts were removed from each pot for dissection. The results of the dissections are shown in Table I (page 63).

Field Experiments.

In the Spring of 1935, a rectangular plot of land 100 feet broad and 590 feet long was divided into four equal rectangular plots; two diagonally opposite plots were sown with maize, and the other two with grasses. The grass mixture was intended to include a large proportion of meadow grass, but unfortunately, owing to the sandy nature of the soil and the drought during the summer, this died out quickly, leaving little else but rye grass. In spite of re-sowings of the original mixture the desired grasses were never successfully established. For this reason the experiment was begun afresh in 1936. This time the field was divided transversely into eight

TABLE I.

Results of Cyst Dissections in Pot Experiments.

Treatment.	Full Cysts per 100.	Three-quarters full cysts per 100.	Half-full cysts per 100.	Quarter-full cysts per 100.	Empty cysts per 100.	Percentage viability.	Percentage Reduction in viability.
Control	21	9	6	20	44	35·75%	—
After white maize	5	9	3	13	70	16·5 %	53·85%
After yellow maize	0	17	1	15	67	17·0 %	52·45%
After meadow grasses	1	9	1	6	83	9·75%	72·73%
After *Alopecurus pratensis*	10	8	9	12	61	23·5 %	34·27%

equal strips so that each was composed of half of what had been a grass plot and half of a former maize plot. Any effects that the previous treatments may have had were therefore equally present in each of the eight plots. In the spring of 1936 samples of soil were taken from each plot. The sampling was done by means of a cylindrical soil sampler 9 inches deep, and two bags of soil, each containing five samples, taken at random, were removed from each plot. Four of the plots were then sown with maize, and the four alternate plots with field lupins. At the end of the growing season the lupins were cut down and the whole plot was ploughed up. Some of the maize had been cut and used for fodder during the summer, the rest was ploughed in. In December soil samples were taken in the same manner as before, and the soil was removed to the laboratory for examination. The soil both in the spring and the winter samples was thoroughly dried, sieved and mixed, and samples of cysts were removed.

The results of dissections of cysts from the spring samples showed, on the average, a fairly uniform viability, though this was rather low. The average for the whole plot was only 33·59 % : the lowness of this figure must be due to the fact that the last crop of potatoes had been grown on this field in 1931, five years previously. A comparison of the viability of the cyst contents on the plots which had been used for the unsuccessful maize and grass experiment of 1935 shows little difference between the four plots. This is shown by the values given in Figure 1. The figure for the viability of the cysts on each plot is the percentage " fulness " found on dissecting 400 cysts.

Maize 1935 34·75%	Grass 1935 39·5%
Grass 1935 26·62%	Maize 1935 33·5%

Fig. 1. Percentage viability of cysts from field plot. Spring 1936.

The arrangement of the plots for the 1936 trials, together with the calculated viability of the cysts on each plot, are shown in Figure 2 (p. 66). The value for the viability was obtained by the dissection of 200 cysts.

Plots numbers 1, 3, 5 and 7 were sown with maize, and numbers 2, 4, 6 and 8 with field lupins.

TABLE II.

Maize.					Lupins.				
Plot No.	% Viability before crop.	% Viability after crop.	Difference.	% Decrease.	Plot No.	% Viability before crop.	% Viability after crop.	Difference.	% Decrease.
1	42·5	22·37	−20·13	47·35	2	32·75	30·87	− 1·88	5·74
3	33·62	27·0	− 6·62	19·69	4	39·62	25·87	−13·75	34·7
5	35·62	28·0	− 7·62	21·4	6	23·12	22·62	− 0·5	2·16
7	32·37	16·12	−16·25	50·19	8	29·12	22·87	− 6·25	21·46
Average	36·03	23·37	−12·65	34·66	Average	31·15	25·56	−5·59	16·01

TABLE III.

Plots.	Full Cysts per 100.	Three-quarter full cysts per 100.	Half-full cysts per 100.	Quarter-full cysts per 100.	Empty cysts per 100.
Nos. 1, 3, 5 & 7. Spring	26·4	7·12	5·75	5·75	55·0
Ditto. Autumn	14·1	7·25	4·5	6·25	67·9
Nos. 2, 4, 6 & 8 Spring	21·9	5·87	6·6	6·25	59·4
Ditto. Autumn	15·9	6·0	7·0	6·5	64·5

1.	42·5 %
2.	32·75%
3.	33·62%
4.	39·62%
5.	35·62%
6.	23·12%
7.	32·37%
8.	29·12%

Fig. 2. Percentage viability of cysts for each plot. Spring 1936.

The results of the pre-crop and post-crop dissections, with the differences between them are shown in Table II (page 65).

It will be seen that the results for the individual plots receiving the same treatment are not very constant, and that variations in the percentage decrease in viability are wide. One factor which probably contributed to this was the variation in growth on the different plots. The maize in particular was very patchy on one or two of the plots. For this reason the average results are of more value than the individual figures. If the average percentage decrease in cyst contents for the two series of plots is compared, it will be seen that on the maize plots it is more than twice that on the lupin plots.

It is interesting to compare the numbers of cysts in the different categories before and after the treatments. The number of full cysts per 100 cysts had decreased more after maize than after lupins, while the completely empty cysts showed a correspondingly greater increase. In the other categories the changes are less marked. (See Table III, page 65.)

Discussion.

It appears, both from pot and field experiments that, when maize is grown in soil containing cysts of the eelworm *H. schachtii*, a decrease in the number of unhatched eggs in the cysts is brought about. In field tests this decrease is shown to have been more than twice as great as that which occurred when lupins were grown : in pot experiments the decrease is seen to have been less than that which was brought

about by the growth of meadow grass, but considerably more than when *Alopecurus pratensis* was grown. It was to be expected that a greater reduction in the viability of the cysts would be shown in pot experiments than in the field, since, in the former case, the roots of the plant under consideration spread in all directions through the whole bulk of the soil used, whereas in the latter, much of the soil is unaffected by the roots. This would account for the fact that a reduction of 53·15 % was brought about by growing maize in plant pots, while in the field the reduction was only 34·66 %. The effect on the cyst contents of growing meadow grass in pots was very striking, the viability of the cysts being reduced by three-quarters.

It is interesting to compare the results of the field experiment in Bedfordshire with those obtained by Triffitt in Yorkshire. Too much stress must not be laid on such a comparison, since cyst population and initial viability were quite different in the two cases. It can be said, however, that the growth of maize on infected soil in Bedfordshire produced a greater reduction in one season in the viability of the cysts than did the growth of a grass mixture in Yorkshire. The reduction in Yorkshire was 23·27 % as compared with 34·66 % after maize in Bedfordshire.

Although maize has been recorded (Hollrung 1890) as a host of *H. schachtii*, it was not found to be attacked by the potato strain of the eelworm in this experiment in spite of a repeated search for cysts. Roots of maize plants which were grown for 29 days in pots of soil infected with the potato strain of *H. schachtii* were stained and examined for larvae, but none were found. Potatoes grown at the same time contained numerous larvae in the roots after only 11 days growth. A few nematodes were found in the roots of maize plants growing on the experimental plots, but these, on examination, proved not to be *H. schachtii*. Thus, although hatching appears to have taken place from cysts in proximity to growing maize plants, the larvae did not enter the roots of the maize.

From the foregoing experiments it appears that maize should prove a useful crop to grow on potato sick land. In one season it is capable of reducing the numbers of unhatched eggs in the cysts by about one third. If no stimulating crop is grown, cysts of *H. schachtii* may remain viable in the soil for many years, so that it is impracticable to attempt to get rid of the parasite by starving it out. By means of such crops as maize

or meadow-grass, however, the starving out of this nematode might become a practical proposition. The chief danger in the method lies in the presence in the soil of the hatched larvae ; since these *do not enter* the maize roots, they must therefore remain free in the soil, and if potatoes are planted before they die out, they would produce a heavy infection on the potatoes, and so nullify the good done by the cultivation of the stimulating crop. As free larvae of *H. schachtii* can survive the winter in soil in the absence of host plants, potatoes should not be grown in the year following maize. If, therefore, maize is to be used on potato sick land in a rotation in which potatoes are grown once every four or five years, it should follow the potato crop as closely as possible, and at least two winters should elapse before potatoes are again grown, in order to ensure that the free larvae shall die out.

The pot experiments seemed to indicate that the meadow grass was more effective in causing hatching of eelworm larvae than maize. On a field scale meadow grass has the advantage that it covers the ground much more closely than maize, and can be allowed to grow continuously for as long as desired, whereas the growing season of maize lasts only five or six months at the most. It is advisable, however, for the ground to be broken up during the winter so that as many of the free larvae as possible may be destroyed by exposure to frost and drying.

Acknowledgment.

The experiments which were undertaken were planned and carried out in conjunction with Dr. M. J. Triffitt, and the writer gratefully acknowledges her ever ready help and encouragement throughout the work. Thanks are also due to Mr. T. H. Ream, farmer at Little Sutton, Beds., on whose land these experiments were carried out and by whom the original observation was made that a good potato crop was obtainable from eelworm infected land on which maize had been grown previously.

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An eelworm infested field showing the marked effect on potatoes of Maize grown, in the preceding year, on the left half only.

To face p. 68.

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The Survival of Free Larvae of *Heterodera schachtii* in Soil.

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It is a well known fact that *Heterodera schachtii* in the brown cyst stage can survive in soil for several years in the absence of host plants. Fuchs found living contents in five-year old cysts of the beet strain of this nematode, while eight-year old cysts of the potato strain have been found by the writer still to be infective to the host plant. Thus, the starving out of this parasite by withholding susceptible crops would appear to be impracticable.

Since the destruction of the contents of the cysts while still within the cyst wall has proved to be a difficult matter, attempts have been made to cause the larvae to hatch, and then to kill them. Hatching may be brought about either by chemical or by biological means. Chemical stimulation has been used by several workers in the past, and more recently by Nebel, Rademacher, Molz and Goffart working on the beet strain, and by Smedley working on the potato strain. Experiments have been made with substances which cause the larvae to hatch and then kill them before they find a plant host. It is possible, however, that after the use of such chemicals, some larvae would survive and remain free in the soil for a time, ultimately dying of starvation if no host could be found.

The biological methods of control of eelworm are based on the same principles as the chemical methods. Trap-crops were employed by Kühn for combating the beet strain, and later adapted for use against the potato strain by O'Brien and Prentice. These workers suggested that potatoes should be grown on infected land for about 50 days : this would be long enough for the roots to become infested with larvae, but not long enough for new cysts to develop. The plants have then to be removed from the soil and destroyed, together with the nematodes which are present in the roots. Since hatching of larvae from the cysts probably continues to a greater or less extent during the whole growing period of

the host many free larvae, which have not had time to reach the potato roots, may be left behind when the trap-crop is removed from the soil. Further crops of the host plant must therefore be withheld until these have perished.

The excretions from the roots of certain grasses have been shown by Triffitt to cause hatching of the potato strain of *H. schachtii*. In a field experiment this author found that when a suitable grass mixture was grown on infected land, the viable contents of the cysts were reduced by 48·63 per cent. in eighteen months. As far as could be ascertained the grasses had not been attacked by the eelworm, therefore it must be assumed that the larvae, on hatching, wandered freely in the soil.

In connection with these methods of control of *H. schachtii* it is obviously important to ascertain how long free larvae of this parasite can live in the soil. Early workers believed that they could not survive the winter. Fuchs has stated that, although at 6°C larvae still live, at this temperature they cannot long survive as movement is very slight and they would be unable to penetrate roots. Reinmuth observed very slight movements in larvae of the potato strain after they had been for 13 months in tap water and in snow water. Some of his experiments were carried out at room temperature and some at a mean temperature of 20°C. In each case the *contents* of 20 cysts were used. It is thus possible that the larvae he observed had not existed as such during the whole of the 13 months, but had hatched from the eggs during that time. Moreover Reinmuth did not determine whether, after 13 months existence in water, the larvae were capable of entering the roots of a host plant.

It is asserted by Nebel that larvae of the beet strain of *H. schachtii* can survive for two years in soil in which no plants are growing. He infected soil in plant pots with hatched larvae, and after varying lengths of time he grew beet plants in the soil and examined the roots for larvae. He found these present 379 days after the commencement of the experiment. The number found after this period was about 50 per cent. of that observed during the first few weeks of the experiment. From this he concludes that larvae would be found living in the soil for another year, having existed two years in soil in which no plants were growing.

To obtain more exact information on the survival of free larvae of the potato strain of *H. schachtii* in soil free from plants, the following experiments were made.

Experiments.

At the end of September 1935 three large plant pots were filled with soil which was known to be free from the potato strain of *H. schachtii*. Two of these, A and B, each containing about 4,000 cc. of soil 18 cms. deep, were sprinkled with water and freshly hatched larvae from potato strain cysts, to the number of about 3,500 larvae per pot. The third pot, C, holding about 3,000 cc. of soil 14 cms. deep received 21,500 larvae on October 3rd. A and B thus had an average of one larva to every 1·5 cc. of soil, while C had 7 larvae per cc. Pot A was sunk in the ground out-of-doors, and kept free from weeds; B and C remained indoors in an unheated room, where they were kept moist. At monthly intervals a quantity of soil was removed from pot B in such a way that soil from both the surface and the deeper layers was obtained. This was put into two small plant pots, and in each a rooted potato sett was planted. When the setts had been growing in the infected soil for from 6 to 11 weeks the roots were examined for cysts. Soil from the outdoor pot A was tested in the same way but at intervals of two months. The more heavily infected pot, C, was kept for later examination. The idea of examining the soil by the Baermann technique was discarded, since, were larvae to be found, it would be impossible to tell whether they had enough vitality left to invade plant tissues.

Unfortunately the setts planted in November were killed by frost before they had made much root growth, and had to be abandoned. Those planted in October and in December bore no cysts after 9½ weeks and 12 weeks growth respectively. They were therefore fixed and stained and examined microscopically for earlier stages of the eelworm. Flemming's solution was used in some cases, and in others acid fuchsin in lactophenol, the latter method having the advantage of being the more rapid. An examination of the stained roots of the two plants planted in October in soil from the indoor pot showed in one case two first stage larvae and two young females, and in the other case three first stage larvae. One of the setts planted in December had one first stage larva and one second stage larva in the roots, but nothing could be found in the roots of the other. Potato setts planted in soil from pot A and pot C in February 1937, over 16 months since the infection of the soil with larvae, were examined after three weeks' growth. No larvae of *H. schachtii* could be found in the roots of the plant which had been growing in soil from the outdoor pot,

but 5 larvae were found to have invaded the plant growing in soil which had been kept indoors.

Table I shows the results in detail.

TABLE I.

Larvae added to soil. Sept. 27th 1935.

Date of planting setts in infected soil	Time since addition of larvae to soil	Plants growing in soil kept out-of-doors Pot A		Plants growing in soil kept indoors Pots B and C	
Jan. 7th 1936	3 months	1 cyst	5 cysts	3 cysts	2 cysts
Feb. 3rd ,,	4 ,,	—	—	2 cysts	1 cyst
Mar. 2nd ,,	5 ,,	2 cysts	1 cyst	1 cyst	3 cysts
Apr. 1st ,,	6 ,,	—	—	5 cysts	2 cysts
May 1st ,,	7 ,,	1 cyst	3 cysts	5 cysts	7 cysts
June 2nd ,,	8 ,,	—	—	5 cysts	0 cysts
July 1st ,,	9 ,,	2 cysts	0 cysts	3 cysts	2 cysts
Aug. 1st ,,	10 ,,	—	—	2 cysts	2 cysts
Aug. 28th ,,	11 ,,	0 cysts	0 cysts	4 cysts	0 cysts
Oct. 1st ,,	12 ,,	—	—	2 first stage larvae 2 young females	3 first stage larvae
Nov. 4th ,,	13 ,,	Plant died	Plant died	Plant died	Plant died
Dec. 2nd ,,	14 ,,	—	—	1 first stage larva 1 2nd stage larvae	0 larvae
Feb. 23rd 1937	16 ,,	—	0 larvae	—	5 first stage larvae

Climatic conditions during the winter of 1935–6 were never very severe, and although the soil in the outdoor pot was subjected to frost on several occasions, it was probably never frozen to the bottom of the pot. There was less frost during the winter of 1936–7. During the summer of 1936 the soil was never thoroughly dried out. It is not known to what depth in the soil larvae can descend to avoid cold and dessication, but in all probability field conditions would afford them better protection than is available in experimental plant pots.

It will be seen that the numbers of cysts found on the roots of plants grown in soil from pot A show a gradual decrease during the year, until, after 11 months, no cysts could be found when the plants had been growing

for $13\frac{1}{2}$ weeks. The larvae kept under the less severe indoor conditions appear to have survived longer and in greater numbers than those kept out-of-doors. In this soil, the largest number of cysts were formed on the potato roots during May, and thereafter the number diminished, though larvae were still alive and capable of entering the host after 16 months free in the soil. It is interesting to note that, after 14 months in the soil, although larvae penetrated the roots of the potato plant, no cysts were formed during $9\frac{1}{2}$ weeks. It may be that the larvae, having lost vigour, were slow in reaching the roots ; or, having once entered the roots, they were unable to mature, or very slow to do so. The low temperatures in December when the plants in question were growing may have been responsible for the delay in development of the larvae.

Discussion.

It has been shown that hatched larvae of the potato strain of *H. schachtii* can survive for at least 9 months in soil in which no plants are growing, under climatic conditions such as those experienced out-of-doors during the winter of 1935–6 and the early summer of 1936. Under less rigorous conditions, such as might well occur in a cool greenhouse or in a sheltered position out-of-doors, they are capable of living for at least 16 months. This fact must be borne in mind when considering methods of controlling the parasite which involve the liberation of large numbers of larvae from the cysts into the soil. Under field conditions much greater numbers of larvae than those used in the experiment would probably be present if the stimulating agent were at all efficient. It is not unusual to find an average of about 4 cysts per cc. of soil in potato-sick land. If a powerful hatching stimulant were to be applied to such soil, one would expect at a very low estimate, at least 100 larvae to emerge from the 4 cysts. The soil would thus be very heavily infected with larvae.

Until the hatched larvae have died out in the soil it is obviously important that the host plant should not be grown. Caution should also be applied in growing other plants in such infected soil, since there is reason to suspect that, under certain circumstances, free larvae of the potato strain of *H. schachtii* may be capable of attacking other plants. A case has been recorded by Triffitt (1931) in which carrots were attacked by the parasite when growing in a field where, earlier in the season, potatoes had been ploughed up owing to complete failure. It appears possible that larvae of the potato strain were stimulated to hatch by the presence of

potato root excretion, and then, when the potatoes were removed, being unable to find their normal host, entered an unaccustomed host and reproduced on it. Thus, if possible, soil heavily infected with *H. schachtii* larvae should be kept free from all plants until the larvae have died out. Marcinowski states that the larvae of this eelworm are very sensitive to drying though she does not state what soil moisture content is fatal. The larvae are also killed by prolonged freezing. It would thus hasten the dying out of the larvae in the soil if it were worked in order to expose the lower layers to drying and freezing and to prevent the growth of weeds.

Acknowledgments.

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On the Survival of *Heterodera marioni* Infection Out-of-doors in England.

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Potato tubers infected with *Heterodera marioni* having been received at this Institute, it was decided to attempt to infect a small field plot with this eelworm for experimental purposes. The tubers were planted in the plot but the infection never became established. This was thought to be due to the heavy nature of the soil, since *H. marioni* has been recorded as a field parasite in Britain (Triffitt, 1931), and it is therefore unlikely that climatic conditions were the cause of its failure to become established in this instance. In order to verify this, and to find out how long this parasite can survive in soil in the absence of a host, the following experiment was carried out.

Two large plant pots, of about 4,000 cc. capacity and 18 cms. deep, were filled with soil from a greenhouse where tomatoes which were badly infected with root-knot had been growing. The soil in one pot was carefully sifted to remove all galled tomato roots ; that in the other pot had galled roots broken up and added to it. The two pots were sunk out-of-doors in the plot where the attempt at infection had been made, at the end of October 1935. They were left there and kept free from weeds.

Tomato seedlings were planted in a small quantity of soil taken from each of these two pots at the end of February 1936, 17 weeks after the pots had been set up. After six weeks' growth in the soil galls were found on the roots. The soil was found still to be infective 29 and 39 weeks after the commencement of the experiment. Again in March 1937, 16 months after the infected soil had been placed out-of-doors, tomato seedlings were planted in a portion of the soil from each pot. Three weeks later the roots were stained and examined. In the roots of the plant which had been growing in the soil to which galls had been added, 3 larvae of *H. marioni* were found. The plant growing in the soil from which the

galls had been removed had 7 larvae in the roots. This plant had made better growth and had more roots than the other. In both cases the larvae were found close to the root tips, and the region round the parasite had already begun to enlarge.

Heterodera marioni can thus survive for at least 16 months out-of-doors, and, under the conditions of the experiment, can withstand some freezing, though severe weather was not experienced. This tends to confirm the earlier supposition that the failure of the infection on the Institute's field plot was due to the very heavy nature of the soil.

It is intended to continue the experiment in order to find out how long the nematode will survive out-of-doors in the absence of a host.

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Field Experiments on Control of the "Potato-Sickness" associated with the Nematode, *Heterodera schachtii*.

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Introduction.

Although the "potato-sickness" associated with the nematode *Heterodera schachtii* has been the subject of a good deal of investigation in Britain during the past twelve years, the only practical method of control so far devised is the adoption of a well-planned crop rotation in which potatoes are omitted for several years. The limitation of a general application of this method to the thousands of acres of land infected with this eelworm disease but otherwise admirably suited for the growing of potatoes has led to insistent demands for further study into chemical treatment of affected soil. In the present investigation, comparative control trials of soil treatment by certain chemical substances were carried out on land known to be thoroughly "potato-sick" and the last potato crop grown in it had been a failure. Most of the experimental treatments had already shown in other field experiments some measure of protection from "potato-sickness," at least, judging by the healthier appearance of the foliage and increase in yield of crop. Although the results of previous experience indicated that there was a positive association between application of these various soil dressings and intensity of disease, it was felt that a more accurate computation of the latter, by measuring the eelworm cyst content of the soil, was desirable before any definite conclusion might be drawn. It was decided, under the circumstances, to arrange a number of plots on badly affected ground, make observations on the growing haulms throughout the season, weigh the produce of each plot separately and compare yields with the original and final degree of infestation as measured by cyst counts.

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rendered in connection with the field work involved in the present investigation. Special thanks are also due to Mr. L. T. Lowe, B.Sc., of the Monmouthshire Agricultural Institute, for carrying out the chemical analysis of the land in which the comparative control experiments were conducted.

Technique Adopted for Determination of Cyst Population of Soils.

The field technique adopted in the present investigation for obtaining soil samples to determine the cyst population of the various plots consisted in taking thirty borings of soil with an auger to a standard depth of seven inches at random over each plot. The borings were mixed together to form a bulk sample. For the purpose of cyst estimations at least, the surface soil is far from homogenous, and this method effectively smoothed out the grosser inequalities. The bulk samples thus obtained were air-dried, lightly pulverised and sifted, using a sieve of 2 mm. mesh. The material passing through the sieve was taken as the portion in each case in the examination for cysts. The sieving was considered imperative since the presence of material greater than 2 mm. in diameter would introduce a considerable error in the determination of the cyst content of the different samples of soil. For cyst counts, ten samples of 25 gm. each were drawn by the usual method of quartering, from each bulk sample. The cysts were removed from the soil by the method described by Morgan (1925). The sample of soil was placed in a narrow-necked flask, and shaken with about 400 cc. of water for two minutes. The flask was then filled with water and allowed to stand until the cysts together with the undecayed organic matter had floated to the surface. The floating material was then transferred to a filter paper previously placed on a small sieve and the cysts counted under a low power binocular microscope. To safeguard against the inclusion of a varying number of non-viable cysts in the counts, some were critically examined from each sample of cysts but in all cases living larvae were obtained. It may therefore be safely assumed that the proportion of empty cysts included in the counts was negligible.

It is noteworthy that the accuracy attained in the technique adopted in the present investigations for the determination of cyst content of soils was of a high standard in view of the fact that three-quarters of the counts shown in any vertical column in Table II are within 4 cysts of the mean. Moreover, it may be mentioned that in a technique-test, repeated

counts on soil samples taken subsequently from Plots 1a and 1b at Blackwood gave cyst concentrations for these plots of identical values with those shown in columns 5 and 6 on Table II.

Review of Previous Work on the Chemical Treatments Used.

A retrospect of previous work involving chemical treatment of land infested with *Heterodera schachtii* revealed that sulphate of potash, calcium cyanamide, ferrous sulphate and ferric oxide were the only substances sufficiently promising for application on a field scale to merit further studies. For this reason these chemical substances were included in the present investigation in addition to paradichlorobenzene which had not hitherto been tested under field conditions in Britain.

1. *Potash.*

In 1925 muriate of potash was included by Morgan in the first series of field experiments in Britain designed to test the effects of various chemical treatments on " potato-sick " soil. It was applied at the rate of 5 cwt. to the acre. The treatment led to better growth of the plant but no definite conclusion was possible with regard to yield of crop in view of the wide discrepancy between the original plots and their duplicates in this respect. In the same year Harper-Gray obtained at Warkworth, Northumberland, a distinct improvement in growth, following the application of 3 cwt. per acre of sulphate of potash, whereas, in 1934 he found no benefit at this centre from dressings of potash. Trials by Edwards in 1934, on the other hand, showed an increase in yield of crop at the rate of 6 tons to the acre in Staffordshire after treating infected land with 10 cwt. of sulphate of potash per acre. Blenkinsop described in 1935 a remarkable improvement both of foliage and yield of tubers in Devonshire, where affected soil had been given 12 cwt. of sulphate of potash per acre in addition to 3 cwt per acre which was present in the compound fertiliser added to both the potash-treated plot and the control. Data is presented to show that this treatment produced an increase in yield at the rate of 12 tons 8 cwt. to the acre and that the beneficial results were consistent with the conception of an unbalanced phosphate-potash ratio on the untreated plot.

In 1935 Hurst & Triffitt gave an account of series of laboratory and pot experiments planned to determine whether the addition of certain artificial fertilisers to affected soil modified in any way the rate of liberation

of larvae from the cysts of *H. schachtii*. The investigators concluded from these tests that neither muriate nor sulphate of potash show promise of application on a commercial scale to the problem of ridding infested land of the eelworm.

2. *Calcium cyanamide.*

In the 1929 field experiments carried out by Edwards in South Lincolnshire, it was shown that this fertiliser might prove of some considerable value in reducing losses caused by "potato-sickness." In these trials an increased yield on an acreage basis of 2 tons resulted from an application at the rate of 10 cwt. to the acre. A year later Miles reported a marked improvement in the crop following its use in an experiment conducted in Lancashire. From the outset the plants made good growth and the amount of haulms was in direct relation to the amount of cyanamide applied, namely, dressings varying from ¾ cwt. to 6 cwt. per acre. There was an apparent gain in yield on an average of 2·1 tons to the acre. In 1933 Davies recorded a good crop of potatoes in Caernarvonshire where the land had received cyanamide for the previous three years. Hodson in the following year obtained beneficial results in Cornwall from an application of 6 cwt. per acre while Edwards in the same season found that this amount had no effect in Staffordshire.

It was shown by Hurst & Triffitt in 1935 as a result of laboratory tests and pot experiments that calcium cyanamide is capable of destroying the parasite but very heavy dressings would probably be necessary to give satisfaction in the field. Even in pot experiments, where the material was thoroughly mixed with the soil and the amount of moisture regulated, a dressing equivalent to at least one ton per acre was found essential to reduce the cysts to a minimum.*

3. *Paradichlorobenzene.*

The only reference in literature to research on the treatment of soil with paradichlorobenzene for the control of the potato eelworm, at least in the British Isles, is to Carroll who included it amongst other substances in pot experiments recorded in 1933. The results were negative but the amount of material used in these trials is not stated.

* A detailed account of Field trials with heavy dressings of Cyanamide was published by Hurst and Franklin in the Journal of Helminthology, January, 1937.—Editor.

4. *Ferrous sulphate and Ferric oxide.*

It was shown in 1935 by Hurst & Triffitt in laboratory experiments that solutions of ferrous sulphate when used in sufficient concentrations has a lethal action on the larvae of *Heterodera schachtii*, and that the addition of ferric oxide to a solution of potato-root excretion containing cysts delays the commencement of hatching of the larvae of this nematode. Promising results also followed the application of these substances in the field, judging by the healthier condition of the plants and the increased yield of tubers when compared with the untreated plots.

General Lay-out of the Experiments.

The experiments were carried out in three different districts and in each case on allotments that had been rendered completely valueless for the growing of potatoes on account of " potato-sickness ". All the allotments selected for the purpose had produced excellent yields of potatoes for many years and were still in high state of fertility, judging by the general appearance of the various crops grown on them for the two seasons previous to the experimental treatments described in this communication and also by the analytical survey of the soils. The following figures give the chemical composition of the soil at the three centres :—

	Newbridge	*Blackwood*	*Dowlais* (East)
*p*H	7·3	7·4	6·2
Organic matter	9·65%	5·10%	7·80%
Total Nitrogen	0·43%	0·27%	0·38%
Available Phosphoric acid (P_2O_5)	0·066%	0·047%	0·039%
Exchangeable Potash (K_2O) ...	0·011%	0·012%	0·024%
Carbonates (as $CaCO_3$)	0·18%	0·18%	0·065%
Lime requirement ($CaCO_3$) ...	nil	nil	0·093% or =18·6 cwt. per acre.

These analyses show that there was no deficiency in essential " plant-food " which might account for the failure of the potato crops at these three centres.

The variety of potato used for these experiments was " Great Scot," being certified Scotch seed. It was known that this particular potato is

popular in the areas concerned and one of the principal varieties to suffer greatly on " potato-sick " soils in South Wales and Monmouthshire.

The experimental allotments at all centres were divided into plots of an area 342 square feet, each of which was separated from its neighbour by a path 2 feet wide. The distance between adjacent rows was 2 feet 6 inches and between the " potato sets " 1 foot 3 inches. The potatoes were planted in the first week of May and a dressing of a compound artificial manure at the rate of 8 cwt. to the acre was added to both treated and control plots at all centres. The fertiliser chosen is essentially the standard manurial treatment recommended for potatoes on allotments in the districts involved in these experiments. It consists of 1 part sulphate of ammonia, 3 parts superphosphate and $1\frac{1}{2}$ parts sulphate of potash.

Method and Rate of Application of Experimental Treatments.

The sulphate of potash, calcium cyanamide and paradichlorobenzene were applied at all centres some three weeks previous to planting of the potatoes. They were evenly spread and, immediately after the application, mixed well with the soil to a depth of approximately seven inches by means of spades and forks.

The ferrous sulphate and the ferric oxides were added to the soil, on the other hand, at the time of planting of the potatoes. They were spread in such a way that the bottom and sides of the rows became coated with a fine layer, but care was exercised that the ferrous sulphate did not actually get into contact with the tubers.

The various chemical substances were applied to the respective plots at the following rates :—

1. Sulphate of Potash at the rate of 11 cwt. per acre.
2. Paradichlorobenzene at the rate of $5\frac{1}{2}$ cwt. per acre.
3. Ferrous Sulphate at the rate of 15 cwt. per acre.
4. Precipitated Ferric Oxide at the rate of 6 cwt. per acre.
5. Natural Ferric Oxide at the rate of 6 cwt. per acre.
6. Calcium cyanamide at the rates of 10, 20, 40, 60, 80, and 100 cwt. per acre.

The calcium cyanamide was also applied at one centre (Dowlais) in two dressings. The first application was made three weeks before the potatoes

were planted at the rate of 10 cwt. to the acre and dug in to a depth of about seven inches. The second one was carried out seven days after planting at the rate of 4 cwt. to the acre and raked well into the ground.

Effect of Treatments on the Pathological Condition of the Plants.

Of the three factors taken into consideration to determine the value of the various soil treatments, the pathological condition of the plants is decidedly the least satisfactory since it was not possible to assign to it any standard of measurement. In the present instance, nothing further than the naked eye appearance of the growing haulms and occasional root was practicable. All the experimental plots were kept, however, under constant supervision from the time of planting until the foliage had completely died down. It is not proposed to discuss in any detail here all the results obtained from these general field observations but rather a brief summary of the most outstanding features that appeared to indicate a positive association between the application of the different soil dressings and intensity of disease or growth of the plant.

So far as could be gathered from observations made in the field, growth started quite normally, and little or no difference was apparent on the different plots. An exception must be noted in the case of the plots which received calcium cyanamide in amounts exceeding 40 cwt. to the acre. The plants on these plots were less numerous and decidedly slower in emerging through the soil than those on the controls. This retardation in appearance of the plants above ground was also accompanied by a distinct darker green foliage, restricted root system and a definitely slower rate of growth of the haulms in the early part of the season. The adverse effects were in direct relation to the amount of cyanamide applied being most evident on the plots treated with the heaviest dressing.

In the early part of July a marked contrast in the growth and vigour of the plants could be detected between the various treatments and as the season progressed the differences became gradually more obvious and, by the end of this month, were most pronounced. At this time it was evident that, whatever its ultimate influence might be on yield of tubers or degree of infestation of the roots by the nematode, the calcium cyanamide exerted a decided beneficial effect on the growth of the haulms. At all centres the potato plants on the calcium cyanamide treated plots

were growing vigorously and of a luxuriantly healthy colour whilst those on the controls were stunted in growth and their foliage exhibited the customary symptoms of the "potato-sickness" associated with root invasion by *H. schachtii.*

On the plots treated with ferrous sulphate, the plants showed indications of distress in the early part of the season but they gradually improved somewhat in appearance compared with the controls. Even the best plants, however, could not be considered normal. In contrast to the ferrous sulphate plots, the growth of the haulms on those which received precipitated ferric oxide was, in comparison with that on the controls, slightly more advanced in the early stages of development of the plant. This superior growth was maintained for some weeks but by about the middle of July was no longer evident. At all centres the plots treated with natural ferric oxide produced plants which were not at any time plainly distinguishable from those on the controls neither in amount nor in colour of the foliage. The plants on the paradichlorobenzene plots exhibited, on the other hand, a slight improvement in these respects over the controls. From the outset the plants on the potash plots made good growth and, on the whole, their general appearance in regard to colour and freedom of the haulms from the pathological manifestations associated with "potato-sickness," was highly promising at least for the greater part of the growing season. Nevertheless, at no time did they approach such a high order of resistance to the disease as shown by the potato plants on the calcium cyanamide plots.

The foliage of the plants on the control plots died down at all centres in the first half of August. At this time the haulms on all the calcium cyanamide plots were green in colour and there was an entire absence of symptoms that would lead one to suspect the presence of "potato-sickness" except in the case of the plots treated with the smallest dressing, namely, application at the rate of 10 cwt. to the acre. The haulms on the latter plots showed at this stage rather dull foliage and a considerable proportion of the lower leaves were virtually dead. As in the earlier part of the season the remaining series of plots treated with calcium cyanamide exhibited a great variation in the amount of foliage produced, depending on the rate at which this material had been added. The plots which received a dressing corresponding to 20 cwt. per acre were conspicuously the best both for their luxuriance of growth and number of individual

plants. In appearance the haulms on these plots compared very favourably in all respects with those of healthy potato plants grown under normal conditions on adjacent allotments known to be free from infection. The plants on the plots treated with calcium cyanamide in amounts equivalent to 100 cwt. per acre were, on the other hand, very uneven in size and it was calculated that on the average approximately 35 per cent. of the " potato sets " had completely failed. These results are attributed to the poisonous action of the heavier dressing of the calcium cyanamide on plant growth. Examination of the plots treated with amounts intermediate between 20 cwt. and 100 cwt. per acre revealed that the plants were increasingly susceptible to the toxic effects of this chemical substance as heavier dressings were applied.

The haulms on the ferrous sulphate and the ferric oxides plots also died off at all centres in the first half of August. Those on the paradichlorobenzene plots had not entirely died down at that time but it was evident that very little tuber growth could be expected. The greater part of the foliage on the sulphate of potash treated plots was still green and persisted into the first week of September despite the fact that definite signs of " potato-sickness " had been present from an early date.

The comparative appearance, towards the middle of September, of one of the control plots and an adjacent plot which had been treated with calcium cyanamide at the rate of 20 cwt. to the acre is shown on Plate 1, Fig. 1 and 2.

Effect of Treatments on Yield of Crop.

The entire crop of all plots was lifted in the first week of October and at that time the plants which had received calcium cyanamide in amounts exceeding 20 cwt. to the acre were still carrying large green haulms and had not fully matured. In order to get an accurate determination of yields of crop under the various treatments, the produce of each plot was lifted carefully by hand and separately weighed. The results are tabulated on Table 1, where it can be seen that the degree of " potato-sickness " was of a fairly uniform order throughout the experimental areas at all centres, judging by the yields obtained on the control plots. A further striking feature of the results from an analysis of the weights of crop on the controls is the very low yields obtained from the diseased areas upon which the plots in the present trials were laid. A normal yield fluctuates

about 8 tons per acre, but on the control plots in these experiments the yields varied from less than 10 cwt. to about 2 tons per acre ; the average

TABLE I.
Effect of Treatment on Yield of Crop.

Centre	Treatment	Index of Plot	Per Plot		Per Acre			
			Total Yield	Average Yield	Average Yield		Increased Wt. over control	
Newbridge	Cyanamide per Acre.		lb.	lb.	Tons	cwt.	Tons	cwt.
	Control (untreated)	1a 1b	43·00 39·00	41·00	2	6·7		
	10 cwt.	2a 2b	109·00 98·00	103·50	5	16·3	3	9·6
	20 cwt.	3a 3b	142·00 148·00	145·00	8	7·0	6	0·3
	40 cwt.	4a 4b	112·00 102·00	107·00	6	1·7	3	15·0
	60 cwt.	5a 5b	90·00 82·00	86·00	4	17·8	2	11·1
	80 cwt.	6a 6b	66·00 75·00	70·50	4	0·2	1	13·5
	100 cwt.	7a 7b	56·00 51·00	53·50	3	0·8		14·1
Blackwood	Control	1a 1b	14·00 15·75	14·75		16·8		
	Precipitated Ferric Oxide	2	30·25	30·25	1	14·4		17·6
	Natural Ferric Oxide	3	18·50	18·50	1	1·0		4·2
	Ferrous Sulphate	4	20·00	20·00	1	2·7		5·9
Dowlais (West)	Control	1a 1b	7·50 9·50	8·50		9·6		
	Precipitated Ferric Oxide	2	22·75	22·75	1	5·9		16·3
	Natural Ferric Oxide	3a 3b	16·75 17·25	17·00		19·3		9·7
	Ferrous Sulphate	4	13·25	13·25		15·1		5·5

TABLE 1—*continued.*

Dowlais (East)	Control	1a 1b	23·50 17·50	20·50	1	3·3		
	Cyanamide in Two Dressings	2a 2b	92·00 85·00	88·50	5	0·6	3	17·3
	Sulphate of Potash	3a 3b	65·75 75·25	70·50	4	0·2	2	16·9
	Paradichloro-benzene	4a 4b	49·50 61·50	55·50	3	3·1	1	19·8

yield except at the Newbridge centre was no more than the weight of the seed potatoes planted on the plots.

When the figures for the average yield are considered in relation to the various dressings, which are shown in the fifth column of Table 1, it is evident that all the soil treatments employed had been used with advantage. The greatest increase in yield is found on plots treated with calcium cyanamide at the rate of 20 cwt. to the acre. The average weight of potatoes on these two plots was 145 lb., compared with 41 lb. on the controls, showing an increase in yield due to this treatment on an average of 104 lb. per plot or approximately 6 tons to the acre. The average yield of 8 tons per acre from these plots is considered highly satisfactory in view of the fact that it equalled the weight obtained during the year from some of the best potato crops in the district. The healthy appearance of the plants throughout the growing season and the satisfactory nature of the yield strongly suggest that, under the conditions prevailing in South Wales, the protection afforded by this treatment may assist the plants to overcome entirely the effects of "potato-sickness".

It will also be noted from the figures for the average yield that a definite association existed between the weight of the potato crop and the amount of calcium cyanamide added to the soil. The data shows that the yield of crop increased in proportion to the amount of calcium cyanamide applied when that was not greater than 20 cwt. to the acre and progressively decreased with increasing amounts of this chemical when the rate of application exceeded 20 cwt. per acre. Although a single field trial is insufficient evidence on which to base assumption that calcium cyanamide in dressings under 20 cwt. to the acre is unable to afford complete protection of potato crops from the adverse influences of "potato-sickness," these results, taken in conjunction with the conclusions of Hurst & Triffitt from

laboratory and pot experiments (1935), seem to be of some considerable significance. At the same time it must not be inferred that provided infected land is treated with calcium cyanamide at the rate of 20 cwt. per acre complete alleviation from the disease is assured since the degree of efficiency of this treatment would naturally be closely related to the thoroughness with which the material is mixed and incorporated in the soil. Further, there is reason to suppose from the general field observations made by the writer in different districts in Lincolnshire, Staffordshire and in later years in Wales, that both the physical nature of the soil at the time of application of calcium cyanamide and the climatic conditions directly thereafter also exercise a marked influence on the effectiveness of this fertiliser in diminishing the deleterious action of the disease on the potato plants. In all cases examined, the calcium cyanamide had been applied as a small dressing in no instance greater than 12 cwt. to the acre. It would appear from these examinations that the first essential is land in a good state of tillage and moderately warm and dry at the time of treatment. The dressing should be mixed as intimately as possible with the soil and the application made when there is every likelihood of rain within a few days after the work has been completed. It is noteworthy in this connection that these ideals were adhered to, at all centres in the present investigation and that heavy showers of rain fell in the second and third week from the date of treatment.

The progressive decline in yield of potatoes with successive increased dressings of calcium cyanamide when the rate of application exceeded 20 cwt. to the acre is attributed to the injurious action of the treatment in actually destroying a high percentage of the potato plants before they appeared above ground and in retarding the normal rate of growth of the remainder throughout the earlier stages of their development. It is realised that the toxic results experienced in the case of these heavier dressings might conceivably be due to the fact that the interval between the time of application of the calcium cyanamide and of planting the respective plots with potatoes was only 3 weeks. Such a supposition accords with the findings of Crowther & Richardson (1932) who have shown that the toxicity of calcium cyanamide to plants in the seedling stage falls off rapidly as the interval between adding this material and cropping is increased and is proportional to the amount of cyanamide present during a short period after the seeds are sown.

PLATE I.

Fig. 1. View of potato crop on control plot (untreated) and adjoining that illustrated in Fig. 2

(Photographed 15.9.36)

Fig. 2. View of potato crop on plot treated with calcium cyanamide at the rate of 20 cwt. per acre.

(Photographed 15.9.36)

The general conclusion to be drawn from the figures for the relative effects of the treatments, other than calcium cyanamide, upon yield seems to provide evidence that, while all these chemical substances had benefited the crop, the increase in weight of tubers produced in each instance was generally small and negligible from a practical standpoint except where sulphate of potash had been used. The limitation of the increased yields under these various treatments is attributed to their failure in protecting the plants from the pathological conditions associated with "potato-sickness" rather than to any impaired effects of the materials on the normal growth of the plant. In each case the yield seemed to be in direct relation to the intensity of the disease as manifested during the growing season in the aerial parts of the plants.

Effect of Treatments on the Cyst Content of the Soil.

Since it was desired to compare yields of potatoes from each plot, it was not possible to disturb the roots of large numbers of plants during the growing season for the purpose of ascertaining the influence of the various treatments on the development of the eelworms in the root tissue. Some attention was, however, paid to the relationship of the different treatments to the time required for the protrusion of the female cysts from the roots. Observations were made on the roots of two plants, lifted at random from each plot, on four occasions at intervals of approximately seven days, starting about the middle of June. It is realised that intervals of a week between examinations and only two plants from each plot inspected on each occasion permit a rather large margin of error, nevertheless some useful information has emerged from a consideration of the data collected in this manner.

The practical import of such information is that cystic nematodes became visible to the naked eye on the plants of the untreated plots at all centres about June 24th, that is, approximately 44 days from the time the potatoes were planted. About this date, white stage females were also seen in fair numbers on the plants of the plots treated with sulphate of potash and paradichlorobenzene. A week later some eelworms were discovered to have reached the cyst stage on all the other treated plots except where calcium cyanamide had been used in amounts above 10 cwt. to the acre. On July 8th, newly emerged cysts were discernible on the plots treated with calcium cyanamide at the rate of 20 cwt. per acre as well as on those which had received this chemical substance in two

dressings. The last inspection of the series made on July 16th revealed that, while a few nematodes had succeeded in attaining sexual maturity even where 40 cwt. of calcium cyanamide per acre had been well incorporated with the soil, no cysts appeared to be present when this compound was used at heavier applications. The plants from all the plots which were found infested on the previous occasions showed at this time, on the other hand, innumerable cysts in all stages of development.

From these observations it would appear that the soil treatments tested in these trials except sulphate of potash and paradichlorobenzene had exerted some influence on the development of the nematode, which partially inhibited the formation of cysts on the roots of the potato plant. In all these instances, the addition of the chemical substances to the soil had in some way checked cyst formation of the nematode at least for a short period in the early stages of development of the plants. No conclusions can be safely drawn as to the actual nature of the action produced by the particular chemical used in each case but it may be pointed out that such action might have been due, in the light of results obtained by Hurst and Triffitt from laboratory tests (1935), to (a) retardation in the commencement of hatching of eggs of the parasite or to (b) destruction of its larvae at least for a short interval after planting of the potatoes and before the roots had extended to regions which had not received treatment. That the effect of the ferric oxide in protecting the potato roots from invasion by *H. schachtii* larvae is at the most but temporary in character even where roots are well within the range of its influence in the soil, was clearly shown in the present investigation by the fact that cysts were equally common on the roots thickly coated with a deposit of this red coloured compound as on those entirely free from it. Although the actual roots that had been in direct contact with their respective chemicals could not be detected under the other treatments, it was evident that all roots exhibited essentially similar infestation of a severe nature and were in this respect comparable with those of the plants on the ferric oxides and control plots. An exception must be mentioned, however, in the case of the plants on the plots which had received applications of calcium cyanamide ranging from 60 cwt. to 100 cwt. per acre. At the time of lifting the crop, the plants on these plots were carrying not only large green foliage completely devoid of any symptoms associated with "potato-sickness" but also a high percentage of roots that had remained entirely

free of cyst infestation. Even on the worst attacked roots the majority of the encystic female worms were still in the white stage of their development. The absence of cysts on the roots was most marked on the plots where the dressing had been made at the rate of 100 cwt. per acre. Here the infection was restricted to a few roots on an occasional plant. Another remarkable feature regarding the infestation on the plots treated with calcium cyanamide in amounts exceeding 40 cwt. per acre was that, in general, the cysts were comparatively rare on the proximal few inches of root, but as the roots penetrated into the deeper soil they became badly infested. From this it would seem that when calcium cyanamide is applied in the manner adopted in these experiments, its influence even in very heavy dressings is on the whole limited to the upper few inches of soil, and that, although most of the surface roots may be protected from severe invasion by the parasite, the remainder of the root system needs a more complicated method of treatment. This would probably entail the use of a special tillage machine for the intimate incorporation of the material with the soil to a depth approximately equal to that penetrated by the roots of the crop.

From an examination of the detailed figures for the cyst concentration of the soil before and after cropping on Table II, it is evident that none of the treatments used had succeeded in reducing the eelworm population except in the case of calcium cyanamide when applied at the rate of 100 cwt. per acre. The cyst content of the two plots treated in this manner had decreased on an average from 54·7 to 29·4 cysts per 25 gm. of soil, that is, the eelworm population of the soil had been reduced by this treatment between March and October, by 46·25 per cent. This figure, indicative of reduction in eelworm infection, is still more striking when compared with the average cyst count of the soil samples taken after cropping on the control plots at the same centre. On these latter plots the infestation was found to have been increased on an average basis from 49·9 to 127·3 cysts per 25 gm. of soil, that is, the nematode population had nearly trebled as the result of cropping with potatoes for a single season.

Next, it is significant that, while all the other treatments had failed to effect any reduction in the number of cysts present originally in the soil of the various plots, dressings of 80 cwt. of calcium cyanamide per acre had produced an appreciable relief by comparison with the cyst counts

of the control plots at the end of the growing season. On this basis, an application of 80 cwt. of calcium cyanamide to the acre had decreased the eelworm infection in the soil, on an average, from 127·3 to 55·4 cysts

TABLE II.

Effect of Treatments on the Cyst Content of the Soil.

Centre	Treatment	Index of Plot	Crop Wt. Per Plot	Cysts per 25 grammes Before Cropping	Cysts per 25 grammes After Cropping
Newbridge	Cyanamide Per Acre.		lb.	*	
	Control (untreated)	1a	43·00	48·4 ± 3·1	126·5 ± 2·9
		1b	39·00	51·4 ± 3·2	128·1 ± 2·5
	10 cwt.	2a	109·00	52·5 ± 2·4	203·7 ± 2·4
		2b	98·00	55·1 ± 1·2	213·6 ± 1·7
	20 cwt.	3a	142·00	54·4 ± 3·1	225·3 ± 1·5
		3b	148·00	56·2 ± 3·6	238·4 ± 1·9
	40 cwt.	4a	112·00	56·6 ± 2·0	127·1 ± 1·4
		4b	102·00	53·5 ± 2·1	120·2 ± 1·3
	60 cwt.	5a	90·00	52·6 ± 1·9	84·2 ± 2·1
		5b	82·00	54·3 ± 1·3	86·0 ± 1·2
	80 cwt.	6a	66·00	53·4 ± 3·3	53·3 ± 1·4
		6b	75·00	54·1 ± 2·3	57·5 ± 2·2
	100 cwt	7a	56·00	55·1 ± 2·1	33·0 ± 1·8
		7b	51·00	54·3 ± 2·8	25·8 ± 1·1
Blackwood	Control	1a	14·00	82·3 ± 1·4	102·6 ± 1·2
		1b	15·75	84·1 ± 1·1	110·1 ± 2·2
	Precipitated Ferric Oxide	2	30·25	80·4 ± 1·6	140·5 ± 1·3
	Natural Ferric Oxide	3	18·50	81·2 ± 1·7	120·1 ± 0·9
	Ferrous Sulphate	4	20·00	79·9 ± 1·9	115·4 ± 1·8
Dowlais (West)	Control	1a	7·50	38·3 ± 0·4	61·1 ± 1·9
		1b	9·50	32·1 ± 0·9	56·3 ± 2·4
	Precipitated Ferric Oxide	2	22·75	36·5 ± 1·8	131·4 ± 1·0
	Natural Ferric Oxide	3a	16·75	31·3 ± 1·5	119·8 ± 0·7
		3b	17·25	37·2 ± 2·4	126·7 ± 0·8
	Ferrous Sulphate	4	13·25	35·4 ± 1·7	114·5 ± 1·1

TABLE II—*continued.*

Dowlais (East)	Control	1a 1b	23·50 17·50	51·1 ± 1·6 49·5 ± 1·1	129·6 ± 1·5 117·4 ± 1·6
	Cyanamide in Two Dressings	2a 2b	92·00 85·00	52·3 ± 2·4 49·2 ± 1·0	192·3 ± 1·4 186·5 ± 2·2
	Sulphate of Potash	3a 3b	65·75 75·25	50·4 ± 2·7 51·9 ± 2·1	189·1 ± 0·9 178·7 ± 1·0
	Paradichloro-benzene	4a 4b	49·50 61·50	50·8 ± 0·6 45·7 ± 1·2	146·8 ± 2·3 156·5 ± 3·1

* Throughout this Table the Standard Error is calculated according to the usual formula, namely :—

$$S.E. = \frac{\sigma}{\sqrt{n}}, \text{ where } \sigma = \sqrt{\frac{s(x-x)^2}{n-1}}$$

per 25 gm. of soil or by comparison with the original eelworm content of the plots so treated, the infestation had remained despite cropping with potatoes essentially the same, at least, from a practical standpoint.

When the figures for the final cyst concentrations alone are taken into consideration as a standard of measurement of results, it would seem that dressings of calcium cyanamide equivalent to 60 cwt. per acre had also been used with a decided advantage. The plots which received this treatment showed on an average 85·1 cysts per 25 gm. of soil compared with 127·3 cysts for the same weight of soil from the controls at the same centre, a reduction of about 33 per cent. Normally, considerable increase rather than reduction in the numbers of cysts on these plots would have been expected owing to the more abundant food supply available in the vigorous root systems of the plants. An average increase in weight of potatoes of 45 lb. per plot or 51·1 cwt. per acre over the controls signifies in itself that a more robust plant and consequently a better root development followed the adoption of this treatment. That the concentration of cysts in the soil of "potato-sick" land is greatly influenced by the extent of the growth made by the potato plant was well exemplified in the present series of experiments. It will be seen from Table II that at each centre except where potatoes were grown in soil treated with the heavier dressings of calcium cyanamide, the amount of eelworm infestation, as measured by cyst counts, was related to the yield of crop, that is, to the vigour of the host plants. Thus the most luxuriant growth and the highest yield of tubers were produced by the plants on the two plots

which received calcium cyanamide at the rate of 20 cwt. per acre. These plants also supported the largest eelworm population, judging by the increase in the number of cysts that had taken place under the different treatments during the growing season. The two plots showed after cropping an average cyst count of 231·8 cysts per 25 gm. of soil against 55·3 cysts before treatment, an increase of approximately five times on the original eelworm content. The crop from the plants on the controls was the poorest at this centre, averaging only 41 lb. of potatoes per plot compared with 145 lb. from those of the plots to which dressings of calcium cyanamide corresponding to 20 cwt. per acre had been added. Measurements of cyst contents showed that the nematode infection of these controls had on the average increased from 49·9 to 127·3 cysts per 25 gm. of soil, a change in the eelworm infestation representing rather less than three times the original finding and decidedly the least in comparison with the figures for the difference in cyst count obtained for the other plots, except where more than 20 cwt. of cyanamide had been used. A similar association also existed between the yield of potatoes and the change in cyst content of the soil at the other centres included in the present investigation. In all cases the cyst concentration increased in proportion to the yield of crop except where the potatoes were grown in soil treated with calcium cyanamide exceeding 20 cwt. to the acre.

It seems therefore from a consideration of the experimental data on Table II, that there was at all centres a marked correlation between the final degree of eelworm infestation, as measured by cyst counts of the soil samples taken after cropping, and the yield of the potato crop obtained on each plot. Alternatively, it may be stated that there was an indubitable correlation between the change in cyst content and the treatments used since the latter had protected the plants to a varying extent as shown by the increase in weight of potatoes on the different plots. From the evidence at present available, it is also obvious that the association between the alteration in cyst census and the treatments tested or the yield of crop produced was independent of the original cyst concentration, and was progressively reduced by increasing dressings of calcium cyanamide when the rate of the application exceeded 20 cwt. to the acre. Further, it is apparent that the association between the treatments adopted and weight of the potato crop lifted in each case was not necessarily dependent upon the final number of cysts present or, alternatively, upon the change in the

nematode population of the soil. These observations lead to the conclusion that under certain conditions potato plants have a high degree of tolerance of *H. schachtii* and can be stimulated to overcome the effects of "potato-sickness" at least to some measure by the use of such soil treatments as were included in the present field studies. In adopting this method of control, it should be borne in mind that a treatment, while providing temporary protection from the adverse influence of "potato-sickness" as exhibited by increased yields of potatoes, may merely aggravate the problem of proper utilization of affected land on account of the consequent building up of a high eelworm population and may also lead to an unbalanced state of the soil as regards the relative ratio of the essential constituents of plant food present. Though admittedly the result of field trials limited to a single season is insufficient evidence in itself on which to base definite conclusions, nevertheless it seems highly probable from a consideration of the data presented in this paper that of the soil treatments hitherto tested, the use of calcium cyanamide in heavy dressings is likely to prove the most satisfactory means of protecting the potato crops from "potato-sickness" and simultaneously clearing infested land of the nematode.

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Studies on the Helminths of India. Trematoda IV.

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Bucephalidæ Poche, 1907.

Syn. Gasterostomidæ Braun, 1883.

The first record of the representatives of Bucephalidæ was made by Rudolphi (1819) who described *Monostomum crucibulum* and *M. galeatum.* The generic names *Bucephalus* and *Gasterostomum* were proposed by Baer (1827) and Siebold (1848) respectively. Ziegler (1883) pointed out that these two genera are identical and that the name *Bucephalus* having priority, *Gasterostomum* falls into its synonymy. At this stage Braun (1883) proposed the family name Gasterostomidæ, but since *Gasterostomum* is a synonym of *Bucephalus,* the family nomenclature Gasterostomidæ lapses into the synonym of Bucephalidæ Poche, 1907. Diesing (1855, 1859) split up the original genus into *Bucephalus* and *Rhipidocotyle,* but this was ignored until very recent times and as a result of this, many species were included in the genus *Gasterostomum.* Odhner (1905) placed the species having sucker at the anterior end in the genus *Gasterostomum* and proposed the new generic name *Prosorhynchus* for those having a " rhynchus " at the anterior end. Nicoll (1914) studied the classification of this group carefully. He divided the family Bucephalidæ into two subfamilies : Prosorhynchinæ with the genus *Prosorhynchus* and Bucephalinæ with the genera *Bucephalus, Bucephalopsis* and *Rhipidocotyle.* Ozaki (1924, 1928) adopted Nicoll's classification and added the genera *Gotonius, Nannoenteron* and *Dolichoenteron.* Issaitschikow (1928) proposed the genus *Skrjabinella* for the species *P. aculeatus* Odhner, 1905. In the recent years Eckmann (1932) published a comprehensive work on this group. He does not agree with Nicoll in splitting the family Bucephalidæ into two subfamilies on the only basis of a possession of a sucker or a rhynchus at the anterior end. He regards *Gotonius* Ozaki, 1924 as a synonym of *Prosorhynchus* Odhner, 1905, and similarly

he merges *Nannoenteron* Ozaki, 1924 into the synonymy of *Rhipidocotyle* Diesing, 1858. Manter (1934) pointed out that *Skrjabinella* Issaitschikow, 1928 is synonymous with *Prosorhynchus*. At the present moment therefore the valid genera belonging to Bucephalidæ are *Bucephalus*, *Bucephalopsis*, *Prosorhynchus*, *Rhipidocotyle* and *Dolichoenteron*. These could be distinguished from one another with the help of the key published by Eckmann (1932, p. 95).

Bucephalopsis (Diesing, 1855) Nicoll, 1914.

Bucephalopsis karvei n. sp. (Figs. 1–4).

Through the courtesy of Professor J. N. Karve of Poona the writer got an opportunity to examine the material of this species collected on two different occasions from the intestine of *Belone cancila*. The specimens were divided into three different tubes, two of which were collected in 1931 and the third one in 1934. The material was abundant and was in an excellent state of preservation. The members of the genus *Bucephalopsis* are well known for showing a large diversity of variations, particularly in regard to the essential genital glands, and some of these variations are of very large magnitude. For this reason about three dozen specimens were studied, some of which were stained and mounted in balsam and others were only cleared in beechwood creosote. A couple of specimens were studied in serial sections. The following description relates to all the material studied.

The worms are inversely pear-shaped. Both the extremities are bluntly rounded, but the anterior one is broader than the posterior. They measure 0·5–0·965 mm. in length and the maximum breadth which is attained a short distance behind the posterior border of the anterior sucker is 0·27–0·57 mm. The cuticle is covered with minute spines which are noticeable only under higher magnifications. Situated subterminally at the anterior end is a large sucker measuring 0·16–0·246 × 0·142–0·227 mm. It is, as usual, surrounded by noncellular glands. The pharynx surrounding the mouth is fairly large and measures 0·044–0·060 mm. in diameter. It lies centrally on the ventral side, at a distance of about one-third the length of the body from the posterior end. It is followed by a thin and fairly long œsophagus measuring 0·55–0·82 × 0·01–0·014 mm. The œsophagus is slightly swollen

posteriorly and opens into the globular or oval intestine measuring 0·068–0·09 mm. in diameter. The intestinal wall in not thin as in some species, but is fairly thick.

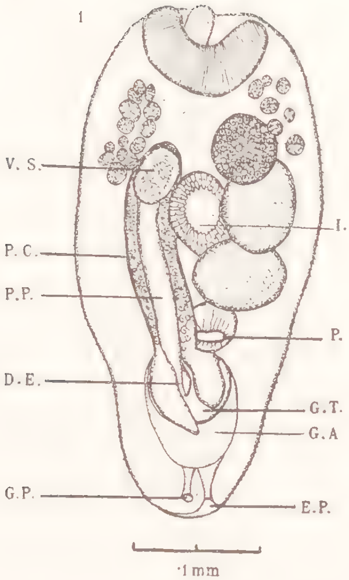
The excretory system consists of the excretory bladder which is an elongated sac extending anteriorly to a distance somewhat midway between the pharynx and the intestine. It measures about 0·284 × 0·054 mm. and opens externally by means of the excretory pore which is situated centrally at the posterior end.

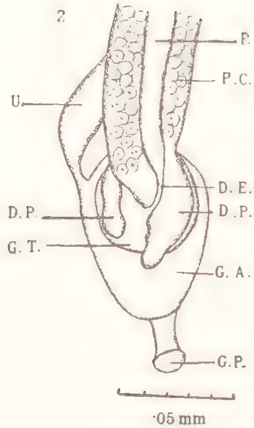
The male genitalia consist of two testes which are somewhat globular or oval and measure 0·108–0·12 × 0·082–0·09 mm. They lie to the right of the middle line close to the saccular intestine. As a rule, the anterior testis is larger than the posterior. Their relative position varies from specimen to specimen. Occasionally one may be seen partially overlapping the other. The cirrus sac and the genital atrium form the most conspicuous parts of the anatomy of the worm and together occupy more area than any other individual organ. The cirrus sac is much elongated and lies to the left of the middle line. It measures 0·235–0·295 × 0·056–0·063 mm. and is approximately half the length of the entire body. It extends from some distance anterior to the saccular intestine to a distance slightly posterior to the pharynx. At its anterior end is situated an oval vesicula seminalis measuring 0·051–0·07 × 0·033–0·05 mm. The terminal portion of the male genitalia projecting into the genital atrium has a somewhat different structure and differs in detail from the corresponding portion of the allied species. On the ventral surface is an almost globular flap which may be termed the " genital tongue." Dorsally are seen two processes which together with the cirrus sac resemble the forearm of a man. On the right side is a small thumb-like process, while on the left is a much larger process which is much wider at the base and terminates in a narrow blunt process. The ductus ejaculatorius opens at the centre of this process on the left side. A small, narrow canal leads from the genital atrium to the genital pore which is situated near the posterior end, close to the excretory pore.

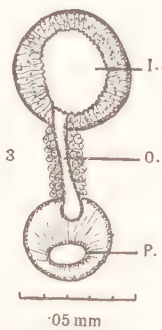
The female genitalia consist of the ovary which is almost round and is situated dorsally, anterior to the saccular intestine, sometimes partially overlapping the anterior testis and the vitelline gland of the right side. It measures 0·056–0·082 mm. in diameter and gives out posteriorly a short, thin oviduct. The shell gland is situated immediately posterior

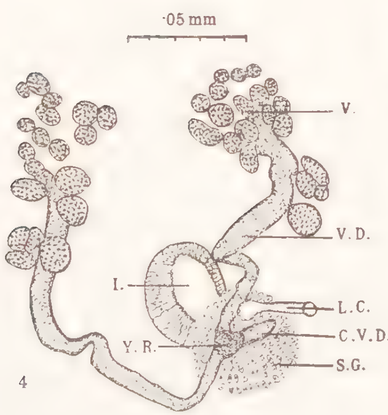
to the intestinal sac or sometimes to its postero-lateral side. The Laurer's canal measures 0·043 mm. in length. The receptaculum seminis is absent. The vitellaria consist of two groups of rounded follicles situated postero-laterally to the anterior sucker. These groups show some variations in regard to the shape. They may be rounded or elongated, but in no case are they disposed in a band-like manner parallel to the sides of the body as in some species of the genus. The vitelline ducts are somewhat sinuous and meet posteriorly to form a vitelline reservoir which opens into the oötype by means of a short duct. The uterine coils are seen for the most part on the left side, passing dorsally to the cirrus sac. The terminal portion of the metraterm is broad and thin. It opens into the genital atrium on its dorsal aspect. The eggs are light brown in colour, oval, operculated and measure 0·018–0·0215 × 0·009–0·013 mm.

The specimen described here possessing a simple sucker at the anterior extremity falls into the genus *Bucephalopsis*. In 1855 Diesing created the subgenus *Bucephalopsis* for the larval form *B. haimeanus*, but this proved to be the larva of *B. gracilescens* (Rud., 1899). Nicoll (1914) raised the subgenus *Bucephalopsis* to a generic rank with *B. gracilescens* as the type. Several species have, from time to time, been included in this genus, but Eckmann (1932) in his recent work reduced the number to only eight species, these being *B. haimeanus* (Lacaze-Duthiers, 1854), *B. exilis* Nicoll, 1915, *B. pusilla* (Stafford, 1905), *B. triglæ* van Beneden, 1870, *B. gracilescens* (Rud., 1819), *B. arcuata* (Linton, 1900), *B. elongata* Ozaki, 1928 and *B. latus* Ozaki, 1928. Of these eight species the description of *B. arcuata* and *B. triglæ* not being adequate, their validity still remains questionable. Subsequently to Eckmann's work, quite recently Verma (1936) described five new species of the genus, *viz.*, *B. fusiformis*, *B. garuai*, *B. magnum*, *B. confusus* and *B. minimus*. Of these the last three are, in the opinion of the writer, synonyms of *B. garuai*. The minor differences pointed out to be existing between this species and the last three can be ascribed either to difference in age or to individual peculiarities. At the present moment therefore the number of valid species of *Bucephalopsis* are only ten. Of these the one described by the writer is allied to *B. haimeanus* and *B. fusiformis* on account of the disposition of the vitellaria and the general form of the body. It can, however, be distinguished from both these species. *B. haimeanus* has more or less connubial or slightly oblique testes, while in the specimen described,









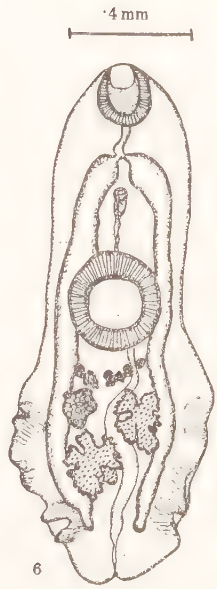


Fig. 1. *Bucephalopsis karvei* n. sp., dorsal view. (Uterus not shown for the clarity of the cirrus sac.)

Fig. 2. *Bucephalopsis karvei* n. sp., terminal portion of the male and female genitalia.

Fig. 3. *Bucephalopsis karvei* n. sp., alimentary canal.

Fig. 4. *Bucephalopsis karvei* n. sp., vitellaria, vitelline ducts, Laurer's canal shell gland, etc.

Fig. 6. *Phyllodistomum sp*. Bhalerao, 1937.

(For explanation of abbreviations see p. 122.)

these organs are situated more or less tandem. The cirrus sac is comparatively smaller and the gut is altogether different in *B. haimeanus*. A comparison of the writer's specimen with *B. fusiformis* Verma, 1936 reveals that the cirrus sac in the former case is always longer than half the length of the body while in the latter case it attains, at the most, only half the body length. The alimentary canal is very different in the two cases. In the writer's specimen the pharynx is more powerful, differently shaped and is situated at the posterior third of the body instead of at the midbody or slightly anterior to it, the œsophagus is much longer and thinner and the saccular intestine has thicker walls. In *B. fusiformis* the ovary is far removed from the testes while in the writer's specimens it is either close to or partially overlapping the anterior testis. Apart from these differences the details of the terminal portion of the male genitalia enclosed in the genital atrium are somewhat different in the two cases. In the writer's specimen, in addition to the usual genital tongue on the ventral side, there are two processes on the dorsal aspect, and on the larger one of these is situated the opening of the male genital duct. It will thus be seen that the writer's specimens are much different from all the existing species of *Bucephalopsis* and for this reason it is considered necessary to create a new species for it, for which the name *Bucephalopsis karvei* is proposed after the donor Professor J. N. Karve.

Specific diagnosis of *Bucephalopsis karvei* n. sp.—*Bucephalopsis* : Body inversely pear-shaped. Length 0·5–0·965 mm. Maximum breadth 0·27–0·57 mm. Cuticle covered with spines. Anterior sucker subterminal. Pharynx large, muscular, situated at about the posterior third of body. Œsophagus long and slender. Intestine almost round, with thick walls. Excretory bladder tubular. Testes almost tandem, to the right, close to intestine, anterior one usually larger. Cirrus sac to the left, larger than half the length of the body. Ovary to the right, antero-lateral to intestine, occasionally partially overlapping anterior testis and vitelline gland of the right side. Vitellaria antero-lateral, compact groups, round or elongated. Uterine coils on the left side of the body. Eggs 0·018–0·0215 × 0·009–0·013 mm.

Host.—*Belone cancila.*

Location.—Intestine.

Locality.—Poona (Bombay Presidency).

With a view to facilitate a ready identification of the valid species of *Bucephalopsis* the following key, which is an enlargement of that published by Eckmann, has been constructed.

Key to the species of *Bucephalopsis*.

1.	Excretory bladder Y-shaped	*B. garuai*
	Excretory bladder tubular	2
2.	Vitelline glands compact	3
	Vitelline glands extending like a band along the sides of the body	5
3.	Testes lying on opposite sides of body	*B. haimeanus*
	Testes lying on the same side of body	4
4.	Cirrus sac longer than half the length of body, pharynx at about the posterior third	*B. karvei*
	Cirrus sac at the most half the length of body, pharynx at about the midbody	*B. fusiformis*
5.	Body elongated, ovary lying close to anterior testis ...	6
	Body more or less round, ovary lying near the right side of body and far removed from testes	*B. latus*
6.	Vitellaria lie in the midbody	7
	Vitellaria in anterior part of body	8
7.	Pharynx at posterior third of body	*B. exilis*
	Pharynx at midbody	*B. pusilla* *B. arcuata*
8.	Pharynx near midbody	*B. elongata* *B. triglæ*
	Pharynx between the first and the middle third of body	*B. gracilescens*

GORGODERIDÆ Looss, 1901.

GORGODERINÆ Looss, 1902.

Phyllodistomum Braun, 1899.

Syn. *Microlecithus* Ozaki, 1926.

Catoptroides Odhner in Looss, 1902.

Yamaguti (1934) pointed out that *Microlecithus* Ozaki, 1926 should be regarded as a synonym of *Phyllodistomum* Braun, 1899. The writer is in complete agreement with this suggestion. Another genus that requires consideration in this connection is *Catoptroides* Odhner. According to Odhner (1910) this genus is characterised by the possession of a discoidal posterior part of the body sharply set off by a groove from a stalk-like anterior portion, genital glands situated close together behind ventral sucker, testes quite or almost symmetrical and vitellaria not anterior to but level with or posterior to ovary. Of these characters, the form of the body is not considered to be of much significance by Odhner. As against this the genus *Phyllodistomum* possesses a leaf-like body not sharply

divided into two distinct regions by a groove, obliquely situated testes and vitellaria in front of ovary. A review of the species that have been so far assigned to the genus *Catoptroides* indicates that, with the exception of the original two species of Odhner, *viz.*, *C. spatula* and *C. spatulæforme*, in none of the species the strict limitations of the genus have been observed. In the species *C. macrocotyle* Lühe, 1909 and *C. lacustri* Lowen, 1929 the ovary is situated posterior to the vitellaria. *C. hunteri* Arnold, 1934, has the testes far removed from the ventral sucker and in *C. lohrenzi* Lowen, 1935, they are almost one behind the other. A similar survey of the species of *Phyllodistomum* reveals that in *P. angulatum* Linton, 1907, and *P. enterocolpum* Holl, 1930, the vitellaria are level with the ovary. The genital glands in the former are very close together and the form of the body in the latter is similar to that of *Catoptroides*. *P. staffordi* fits more appropriately in the genus *Catoptroides* than in *Phyllodistomum*. In *P. kajika* (Ozaki, 1926) the vitellaria are level with the ovary. The form of the body and the genital glands in *P. simile* Nybelin, 1926, and *P. carolini* Holl, 1929, suggest more catoptroidean than phyllodistomid affinity. Moreover, in some cases, the descriptions and figures of the same species given by different authors do not agree. In this connection the species *P. staffordi* and *P. angulatum* may be quoted as examples. In view of this confusion in regard to the strict limitations of the genera *Phyllodistomum* and *Catoptroides* and the variable nature of these forms it is suggested that these two genera be combined into a more comprehensive genus *Phyllodistomum* with the following diagnosis.*

Revised diagnosis of *Phyllodistomum*.—Gorgoderinæ : Body flat. Testes symmetrical or oblique, entire or lobed. Vitellaria immediately behind ventral sucker. Ovary postero-lateral to ventral sucker. Parasites of amphibians and fishes.

Type species.—*Phyllodistomum folium* (Olfers, 1816).

Phyllodistomum shandrai n. sp. (Fig. 5.).

Eight specimens collected in 1926 from the rectum of the frog, *Rana tigrina*, in Bombay were given to me for determination by Professor J. N. Karve of Poona. These on examination proved to be a new species of *Phyllodistomum*.

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* During the passage of this paper through the press it came to my notice that Lewis (1935) arrived at similar conclusion in regard to the synonymy of *Catoptroides* with *Phyllodistomum*.

The worms are flat and elliptical, but the actual form of the body is subject to variation, dependent as it is on the degree of contraction. In a fully extended condition the broadest portion of the worm is at the level immediately posterior to the ovary, from where the body tapers both anteriorly and posteriorly and terminate in blunt extremities. In contracted specimens the widest portion of the body is at the level of the posterior testis. The worms measure 2–3·02 mm. in length and the maximum breadth is 0·78–0·87 mm. The cuticle is devoid of any armature. The mouth is terminal and is surrounded by the oral sucker measuring 0·4–0·46 mm. in diameter. The ventral sucker measures 0·55–0·58 mm. in diameter. The œsophagus is short and measures about 0·14 mm. in length. The intestinal cæca pass posteriorly along the sides of the body and terminate at a distance of 0·33–0·475 mm. from the posterior extremity.

The excretory bladder is tubular. The excretory pore is situated centrally at the posterior end.

The testes are large, deeply lobed bodies and measure 0·47–0·61 × 0·28–0·35 mm. They lie obliquely to each other. The anterior testis is situated postero-laterally to the ventral sucker, almost touching and in some cases slightly overlapping the latter organ. In some cases the anterior testis lies on the left side of the body, while in others it lies on the right. The genital pore is situated centrally, a small distance in front of the ventral sucker. The terminal portion of the male genitalia could be made out only in serial sections. It consists of an oval vesicula seminalis measuring 0·13 × 0·065 mm. This is followed by a very short and narrow pars prostatica surrounded by the prostatic glands. The pars prostatica is followed by a wider and muscular ductus ejaculatorius. The cirrus sac is absent.

The ovary is round or slightly oval and measures 0·265–0·3 × 0·2–0·255 mm. It lies postero-lateral to the ventral sucker, either on the left or on the right side. It is always situated on the side opposite to that of the anterior testis. It lies slightly posterior to the level of the vitellaria. The uterine coils are disposed antero-posteriorly and fill up almost all the space of the body posterior to the ventral sucker. The vitellaria lie on the dorsal aspect and partially overlap the posterior border of the ventral sucker. Each gland is compact and oval. The vitelline ducts emerge from the postero-lateral border of each gland. The shell gland lies

antero-dorsal to the vitelline gland of the side on which the ovary is situated. The Laurer's canal is present, but the receptaculum seminis is absent. The ova are small and measure 0·026–0·028 × 0·0165–0·018 mm.

Of the numerous species of *Phyllodistomum* the form described here is closely related to the species *P. kajika* (Ozaki, 1926). It, however, differs from it in the close proximity of the anterior testis to the ventral sucker, the situation of the testes with respect to the ovary, the posterior extent of the intestinal cæca and the relative position of the vitellaria and the ovary. For these reasons it is considered necessary to create a new species for it for which the name *Phyllodistomum shandrai* is proposed.

Specific diagnosis of *Phyllodistomum shandrai* n. sp.—Body flat, elliptical. Length 2–3·02 mm. Breadth 0·78–0·87 mm. Oral sucker 0·4–0·46 mm. dia. Ventral sucker 0·55–0·58 mm. dia. Intestinal cæca terminating 0·33–0·475 mm. from the posterior end. Testes oblique, deeply lobed, measuring 0·47–0·61 × 0·28–0·35 mm. Anterior testis immediately postero-lateral to ventral sucker. Ovary postero-lateral and close to ventral sucker, measuring 0·265–0·3 × 0·2–0·255 mm. Vitellaria anterior to the level of ovary. Uterine coils filling up the body posterior to ventral sucker. Eggs measure 0·026–0·028 × 0·0165–0·018 mm.

Host.—*Rana tigrina.*

Location.—Rectum.

Locality.—Bombay.

Phyllodistomum sp. (Fig. 6.).

A few specimens from the intestine of *Belone cancila* and *Ophiocephalus marulius* and some from the stomach of *Mastacembelus armatus* collected by Professor J. N. Karve in Poona were given to me for determination. All the specimens are referable to the genus *Phyllodistomum*, but the determination of the exact specific identity was not possible on account of the entire material being immature. On comparison, however, they were found to be closely related to the species *P. macrobrachiola* and *P. superbum.* It is proposed to record here a few observations on the material at my disposal.

The ventral sucker forms a barrier between the anterior and the posterior portions of the body. The anterior portion is elongated and smooth and tapers gradually towards its end. The posterior portion is broad and

possesses crinkled margin. The worms measure 1·07–1·71 mm. in length and the maximum breadth is 0·45–0·64 mm. The cuticle is devoid of any armature. The mouth is terminal and is surrounded by the oral sucker measuring 0·165–0·21 × 0·15–0·18 mm. The œsophagus 0·1 mm. long. The intestinal cæca terminate 0·13–0·17 mm. from the posterior end. The ventral sucker is slightly anterior to the midbody and measures 0·22–0·325 mm. in diameter.

The excretory bladder is tubular and the excretory pore is situated at the posterior end. The bladder describes a characteristic curve in its anterior course.

The testes are deeply lobed, oblique and measure 0·125–0·245 × 0·12–0·20 mm. The genital pore is situated centrally, almost midway between the two suckers.

The ovary is lobed and measures 0·12–0·13 × 0·08–0·12 mm. It is level with the anterior testis and may be situated either on the right or the left side. The vitellaria are situated postero-laterally to the ventral sucker, anteriorly to the level of the ovary.

With a view to facilitate a ready identification of a large number of species now included in the genus *Phyllodistomum* the following key has been constructed.

Key to the species of *Phyllodistomum*.

1.	Posterior part of body more or less disc-like and separated from a comparatively much narrower anterior portion	2
	Posterior part of the body not disc-like and not separated from a narrow anterior part	9
2.	Testes symmetrical or slightly oblique	3
	Testes distinctly oblique	6
3.	Testes in posterior half of discoidal portion	*P. hunteri* (Arnold, 1934)
	Testes at the middle of the discoidal portion	*P. lacustri* (Lowen, 1929)
	Testes in the anterior half of discoidal portion... ...	4
4.	Ovary entire, testes symmetrical	*P. spatula* (Odhn., 1902)
	Ovary lobed, testes slightly oblique	5
5.	Vitellaria level with ovary	*P. spatulæforme* (Odhn., 1902)
	Vitellaria slightly anterior to ovary	*P. staffordi* Pears, 1924

6	Vitellaria level with ovary	*P. patellare* (Sturges, 1897)
	Vitellaria anterior to ovary	7
7	Testes almost one behind the other	*P. lohrenzi* (Lowen, 1935)
	Testes oblique	8
8	Anterior part of body distinctly set off from the posterior part	*P. simile* Nybelin, 1926
	Anterior part of body not distinctly set off from the posterior part	*P. carolini* Holl, 1929
9	Posterior part of body elongated	10
	Posterior part of body leaf-like	13
10.	Testes entire, genital pore far anterior to ventral sucker	*P. marinum* Layman, 1930
	Testes lobed, genital pore near ventral sucker ...	11
11.	Oral and ventral suckers nearly equal	*P. elongatum* Nybelin, 1926
	Ventral sucker nearly one and half times larger than oral sucker	12
12.	Anterior testis close to ventral sucker	*P. shandrai* n. sp.
	Anterior testis much posterior to ventral sucker ...	*P. kajika* (Ozaki, 1926)
13.	Anterior testis obliquely behind ovary	*P. conostomum* (Olsson, 1876)
	Anterior testis level with ovary	14
	Both the testes posterior to ovary	*P. americanum* Osborn, 1903
14.	Testes almost symmetrical	*P. angulatum* Linstow, 1907
	Testes distinctly oblique	15
15.	Oral sucker larger than ventral sucker	16
	Oral sucker smaller than or equal to ventral sucker ...	17
16.	Vitellaria almost digitate	*P. morgurndae* Yamaguti, 1934
	Vitellaria slightly lobed	*P. pearsi* Holl, 1929
17.	Anterior part of body gradually passing into the posterior part	18
	Anterior part of body much narrower than posterior part	20
18.	Vitellaria almost digitate	*P. acceptum* Looss, 1901
	Vitellaria slightly lobed or entire	19
19.	Both the suckers of equal size	*P. unicum* Odhner, 1902
	Ventral sucker larger than oral	*P. linguale* Odhner, 1902

20.	Genital glands large, almost filling up the inter-cæcal space posterior to ventral sucker	21
	Genital glands small and do not fill the inter-cæcal space posterior to ventral sucker	22
21.	Intestinal cæca reach the posterior margin of body ...	*P. megalorchis* Nybelin, 1926
	Intestinal cæca end much anterior to posterior margin of body	*P. parasiluri* Yamaguti, 1934
22.	Testes very close together	*P. fausti* Pears, 1924
	Testes separated by uterine coils	23
23.	Both the suckers almost equal in size	*P. pseudofolium* Nybelin, 1926
	Ventral sucker larger than oral	24
24.	Posterior part of body crinkled	25
	Posterior part of body entire	*P. folium* (Olfers, 1816)
25.	Uterine coils inter-cæcal	*P. macrobrachiola* Yamaguti, 1934
	Uterine coils extend external to intestinal cæca ...	*P. superbum* Stafford, 1904

PARAMPHISTOMATIDÆ Fischoeder, 1901.
PARAMPHISTOMATINÆ Fischoeder, 1901.
Paramphistomum Fischoeder, 1901.
Paramphistomum maplestoni n. sp. (Figs. 7–8.)

A few specimens obtained in 1930 by Dr. P. A. Maplestone in Calcutta from the small and large intestine of the hog-deer, *Hyelaphus porcinus*, were available for study. These on examination proved to be a new species of *Paramphistomum*.

The worms are thick and elliptical and have a tendency to curve ventrally during fixation. They measure 4·05–4·6 mm. in length and the maximum thickness which is attained at about the midbody is 1·1–1·2 mm. The mouth is terminal and is surrounded by the oral sucker measuring 0·465–0·57 × 0·4–0·43 mm. The œsophagus is moderately long and measures 0·2–0·22 mm. in length. The intestinal fork is situated at about the fifth of the body length from the anterior end. The intestinal cæca are fairly wide and pass posteriorly along the sides of the body, terminating at about 0·22 mm. in front of the posterior sucker. The posterior sucker is simple

and measures 0·67 mm. in diameter. The excretory bladder lies dorso-anterior to the ventral sucker and appears somewhat oval in dorso-ventral section. It opens on the dorsal side, almost at the level of the posterior border of the ovary and is situated about 0·147 mm. in front of the posterior end.

The genital pore is situated centrally, at about 0·22 mm. posterior to the intestinal fork. The testes are large, lobed and are situated in the midbody, one behind the other. The anterior one is pre-equatorial while the posterior is post-equatorial. They measure 0·63–0·73 × 0·635–0·77 mm. The vesicula seminalis is a coiled duct and lies in front of the anterior testis, partially overlapping the latter organ. It continues distally into the pars musculosa which is a winding duct measuring about 0·52 mm. in length and 0·075 mm. in width. The pars musculosa is followed by the pars prostatica measuring 0·2–0·31 × 0·12–0·13 mm. and is surrounded by the prostrate glands. The ductus hermaphroditicus is small and opens on the genital papilla. The genital atrium surrounds the genital papilla and communicates to the exterior by means of the genital pore.

The ovary is almost round and measures 0·235–0·32 mm. in diameter. It is situated immediately behind the posterior testis, slightly to the left of the middle line. The shell gland is oval and measures about 0·3 × 0·16 mm. It is situated centrally, posterior to the ovary, partially overlapping the latter. The uterus arises from the posterior aspect of the ovary and its coils pass anteriorly, dorsal to both the testes. The metraterm is thick and measures 0·27 × 0·12 mm. The vitellaria are follicular and very voluminous. They extend from the middle of the oral sucker to the anterior border of the posterior sucker. They are extra-cæcal, cæcal and extend also into the inter-cæcal area. From the level of the genital pore anteriorly and that of the ovary posteriorly the vitelline follicles of either side meet together and are continuous dorsally. The Laurer's canal does not cross the excretory canal as in some cases, but opens in front of the opening of the excretory bladder. The distance between the openings of the Laurer's and the excretory canal is very slight. The eggs measure 0·115–0·119 × 0·05–0·052 mm.

Amongst a large number of species of *Paramphistomum* the one described here has closer affinities to the species *P. orthocœlium* Fischoeder, 1901, but it can be easily distinguished from it by the extent and the

disposition of the vitelline follicles, the position of the testes and the position of the genital pore. For this reason it has been considered necessary to create a new species for its reception for which the name *Paramphistomum maplestoni* is proposed.

Specific diagnosis of *Paramphistomum maplestoni* n. sp.—Length 4·05–4·6 mm. Thickness 1·1–1·2 mm. Oral sucker 0·465–0·57 × 0·4–0·43 mm. Posterior sucker 0·67 mm. in dia. Œsophagus 0·2–0·22 mm. long. Intestinal cæca terminate about 0·22 mm. in front of the posterior sucker. Genital pore 0·22 mm. posterior to intestinal fork. Testes lobed, tandem ; anterior pre-equatorial, posterior post-equatorial. Ovary immediately posterior to hinder testis, slightly to the left of the middle line. Laurer's canal opens in front of excretory canal. Vitellaria extend from the middle of the oral sucker to the anterior border of posterior sucker ; extra-cæcal, cæcal and inter-cæcal. Anteriorly to genital pore and posteriorly to ovary, vitelline follicles of either side meet centrally on the dorsal aspect. Eggs measure 0·115–0·119 × 0·05–0·052 mm.

Host.—*Hyelaphus porcinus.*

Location.—Small and large intestine.

Locality.—Calcutta Zoo.

Paramphistomum cuonum n. sp. (Figs. 9–10.)

Numerous specimens of amphistomes obtained in 1929 by Dr. P. A. Maplestone from the stomach and small intestine of the wild dog, *Cuon dukhunensis*, were available for study. The entire material was in a state of slight degeneration, a point which is indicative of the wild dog not being a proper definitive host of the parasites. It is highly probable that the infection has been acquired by the host by feeding upon some ruminant normally harbouring the parasites. The specimens are, however, very remarkable and for this reason the present opportunity is utilized to give their short description.

The worms are flesh-coloured, flat, thin and translucent. They are elongated and taper gradually towards the anterior end. They measure 3·35–5·7 mm. in length and the maximum breadth, which varies from a fifth to a third of the entire length of the worm from the posterior end, is 0·6–0·95 mm.

The mouth is situated at the anterior end and is surrounded by an oval oral sucker measuring 0·32–0·43 × 0·3–0·32 mm. The posterior sucker

measures 0·47–0·55 mm. in diameter. The œsophagus is 0·46–0·58 mm. long and is surrounded by the œsophageal glands. A definite œsophageal bulb is not distinguishable, but the musculature of the œsophagus immediately anterior to the intestinal fork is more strongly developed. This is more prominent in some cases than others. The intestinal fork is situated at about one-sixth to one-fifth of the body length from the anterior end. The intestinal cæca are moderately wide and pass posteriorly, terminating 0·45–0·72 mm. in front of the ventral sucker.

The excretory bladder appears almost oval in dorso-ventral sections and is situated between the shell gland and the posterior sucker. It measures 0·28 × 0·17 mm. The excretory pore is situated on the dorsal side at a distance of 0·55 mm. from the posterior end.

The genital pore is situated centrally, a small distance posterior to the intestinal fork. The testes are two oval, lobed bodies, lying tandem, in the posterior half of the body. The posterior testis is more or less level with the ends of the intestinal cæca, while the anterior one is close to midbody. They measure 0·475–0·69 × 0·31–0·34 mm. In some cases the lobulated condition of the testes is not distinctly visible, this is particularly so in the immature and more degenerate forms. The vasa efferentia could not be seen, but the male genital duct is differentiated, as usual, into the vesicula seminalis, the pars musculosa, the pars prostatica and a very short ductus hermaphroditicus which opens on the genital papilla projecting into the genital atrium.

The ovary is small and round and measures 0·13–0·23 mm. in diameter. It is situated some distance behind the posterior testis, slightly to the side of the middle line. The shell gland is situated centrally, immediately posterior to the ovary. The Laurer's canal passes anteriorly to the excretory bladder and opens at a distance of 0·9 mm. from the posterior end. The uterus arises posterior to the ovary and passes anteriorly in a winding manner by the side of the testes, occasionally partially overlapping the latter. The metraterm is comparatively wide. The vitellaria consist of a small number of large follicles extending from the genital pore to the anterior border of the posterior sucker. They are extra-cæcal, cæcal and posterior to the ovary, a few follicles are also seen in the inter-cæcal area. The eggs measure 0·120–0·123 × 0·054–0·065 mm.

The species described here is an exception to all the members of the genus *Paramphistomum* in that it is thin and translucent. Anatomically,

however, it is closely related to the species *P. orthocœlium* Fischoeder, 1901, from which it differs in the position of the testes with respect to one another, in the posterior extent of the intestinal cæca, in the nature of the vitellaria, the distance between the openings of the Laurer's and the excretory canals and the position of the genital pore. For this reason it has been considered necessary to create a new species for its reception for which the name *Paramphistomum cuonum* is proposed.

Specific diagnosis of *Paramphistomum cuonum* n. sp.—Body flat, translucent, thin. Length 3·35–5·7 mm. Breadth 0·6–0·95 mm. Oral sucker 0·32–0·43 × 0·3–0·32 mm. Posterior sucker 0·47–0·55 mm. in dia. Œsophagus 0·46–0·58 mm. long. Œsophageal bulb very slightly differentiated. Intestinal cæca terminate 0·45–0·72 mm. in front of posterior sucker. Excretory pore situated 0·55 mm. from the posterior end. Genital pore central, slightly posterior to intestinal fork. Testes oval, lobed, tandem, post-equatorial. Anterior testis near the ends of intestinal cæca, posterior one near the midbody. Ovary round, posterior to hinder testis, slightly to the side of the middle line. Laurer's canal opens 0·35 mm. anterior to the excretory opening. Vitellaria extend from genital pore to anterior border of posterior sucker. Vitelline follicles few and large, cæcal, extra-cæcal and posterior to ovary inter-cæcal. Eggs measure 0·120–0·123 × 0·054–0·065 mm.

Host.—*Cuon dukhunensis.*

Location.—Stomach, small intestine.

Locality.—Calcutta Zoo.

Paramphistomum sp.

Only a single specimen obtained from the stomach of the wild cat, *Felis catus,* near Kasauli was available for study. The specimen had undergone some degeneration and was consequently in an indifferent state of preservation. The degenerate condition of the worm points to the probability that the wild cat is not its definitive host. It is quite likely that the infection may have been acquired by the cat from some ruminant during the process of feeding.

The specimen on examination proved to be a species of the genus *Paramphistomum,* but sufficient details to arrive at the specific identity could not be gathered on account of the unsatisfactory condition of the worm.

The following is a very short account of the parasite.—Body elliptical. Length 2·3 mm. Maximum breadth 1 mm. Mouth terminal. Oral

sucker 0·325 × 0·265 mm. Œsophagus 0·36 mm. long, œsophageal bulb 0·22 × 0·165 mm. Intestinal bifurcation at about the third of the body length from the anterior end. Intestinal cæca are almost straight and terminate on either side of the posterior sucker. Posterior sucker 0·375 mm. in diameter. Genital pore central, immediately behind the intestinal fork. Testes one behind the other, in the central line, slightly lobed and measuring 0·65 × 0·54–0·625 × 0·50 mm. Ovary round, median and in the same line as the testes. Uterus zigzag, passing centrally. Ova not developed. Lymphatic canals a pair on each side. Vitellaria not developed.

Host.—*Felis catus.*

Location.—Stomach.

Locality.—Kasauli (Punjab).

Paramphistomum cervi (Schrank, 1790).

Host.—Neelgai (*Boselaphus tragocamelus*).

Location.—Stomach.

Locality.—Lahore.

Immature *Paramphistomum* sp.

Host.—Hog deer (*Hyelaphus porcinus*).

Location.—Stomach, intestine.

Locality.—Calcutta Zoo.

Cotylophoron Stiles & Goldberger, 1910.

Cotylophoron orientalis Harshey, 1934.

Host.—Sable antelope (*Hippotragus niger*).

Location.—Stomach.

Locality.—Calcutta Zoo.

Fig. 5. *Phyllodistomum shandrai* n. sp.

Fig. 7. *Paramphistomum maplestoni* n. sp., ventral view.

Fig. 8. *Paramphistomum maplestoni* n. sp.: diagram showing the relation of the excretory bladder (E.B.) and Laurer's canal (L.C.)

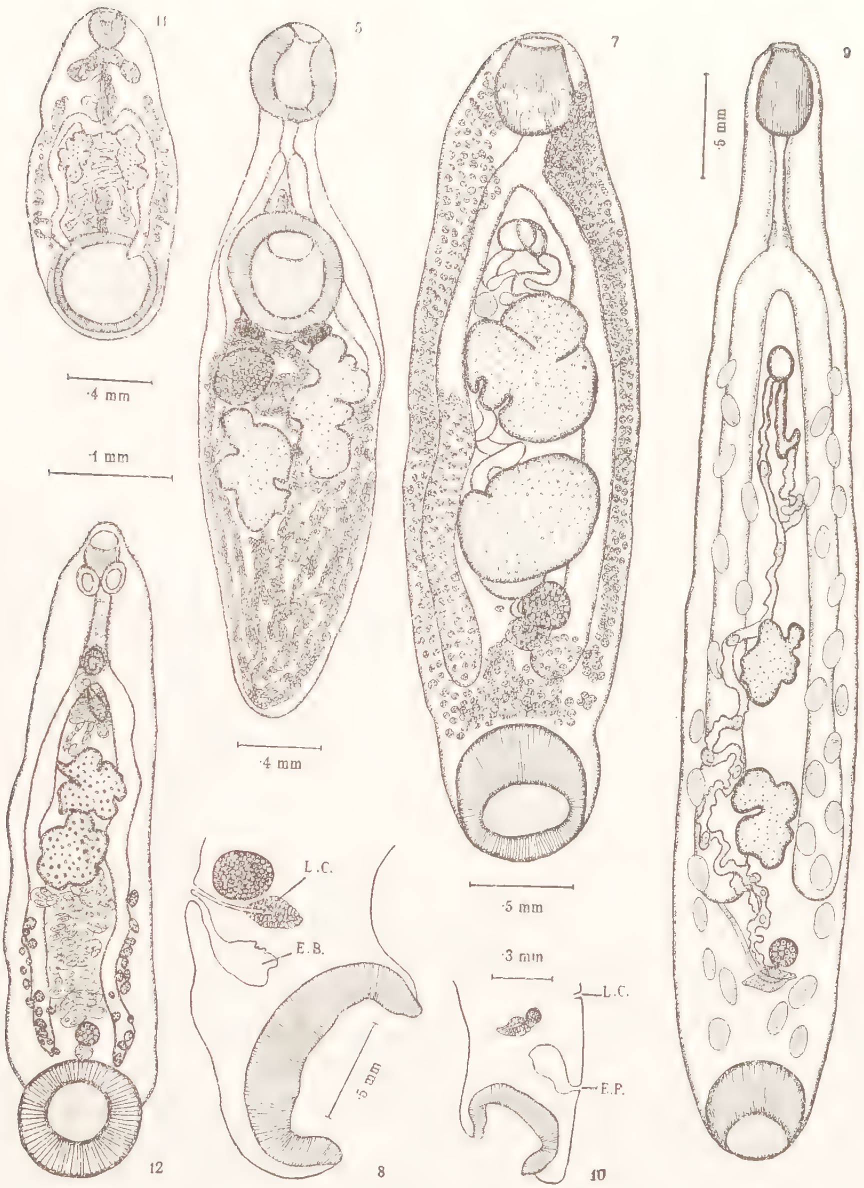
Fig. 9. *Paramphistomum cuonum* n. sp., entire worm, diagram constructed from many slides.

Fig. 10. *Paramphistomum cuonum* n. sp., dorso-ventral section showing the opening of Laurer's canal (L.C.) and excretory pore (E.P.)

Fig. 11. *Helostomatis sakrei* n. sp., dorsal view.

Fig. 12. *Neocladorchis poonaensis*, n. g., n. sp., dorsal view.

(For explanation of abbreviations see p. 122.)


11
5
7
9
·5 mm
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·4 mm
L.C.
E.B.
·5 mm
·5 mm
·3 mm
L.C.
E.P.
12
8
10

GASTROTHYLACINÆ Stiles & Goldberger, 1910.

Gastrothylax Poirier, 1883.

Gastrothylax crumenifer (Creplin, 1847).

Host.—*Boselaphus tragocamelus*, *Hyelaphus porcinus*.
Location.—Stomach.
Locality.—Lahore, Calcutta Zoo.

DIPLODISCINÆ Cohn, 1904.

Diplodiscus Diesing, 1836.

Diplodiscus amphichrus Tubangui, 1933.

About two dozen specimens collected at Bombay in 1926 from the rectum of the frog, *Rana tigrina*, were given to me by Professor J. N. Karve for determination. These resemble in some respects *Diplodiscus amphichrus* Tubangui, 1933 and in others *D. amphichrus* var. *magnus* Srivastava, 1934. A few observations on the material at my disposal are recorded below.

Length 1·32–2·8 mm. Maximum breadth 0·54–0·98 mm. Œsophagus about 0·22 mm. long, œsophageal bulb 0·08 mm. dia. Oral diverticula 0·22–0·25 mm. long. Posterior sucker 0·55–0·9 mm. dia. Accessory sucker 0·135–0·28 mm. dia. Testis 0·65–0·92 × 0·25–0·4 mm. Cirrus sac about 0·13 × 0·10 mm. Genital pore post-bifurcal. Ovary 0·23–0·25 mm. dia. Vitellaria extending from intestinal fork to posterior sucker, not meeting anteriorly in the central line. Eggs 0·122–0·135 × 0·07–0·0715 mm.

CLADORCHINÆ Fischoeder, 1901.

Helostomatis (Fukui, 1929) Travassos, 1934.

Helostomatis sakrei n. sp. (Fig. 11.)

Only a single specimen obtained by Professor J. N. Karve from the intestine of *Labeo calbasu* at Poona was forwarded to me which on examination proved to be a new species of the genus *Helostomatis*.

The worm is almost elliptical with the anterior part slightly attenuated. Both the extremities are bluntly rounded, but the posterior one is slightly broader than the anterior. It measures 1·57 mm. in length and the maximum breadth which is attained at about the midbody is 0·73 mm. The cuticle is smooth and thick. The mouth is situated terminally on

the ventral aspect and is surrounded by the oral sucker measuring 0·185 × 0·17 mm. At its posterior end the oral sucker has two large and stout diverticula which diverge postero-laterally. Each of the diverticulum measures 0·215 mm. in length and is divided into a comparatively thin anterior portion and a bulb-like posterior portion lined with thick cuticle and having powerful musculature. The posterior sucker is large and measures 0·5 mm. in diameter. Its anterior half is bounded by a hood-like margin. Posteriorly it is simple and rounded and is devoid of the " curious, sinuous, puckered outline, with three spout-like projections extending out backwards " as described by MacCallum in 1905 for the species *H. helostomatis*. The œsophagus is 0·3 mm. long and is lined internally with thick cuticle. Posteriorly it has a fairly thick, muscular bulb. The intestinal cæca are characteristically sinuous and terminate dorsally to the anterior face of the posterior sucker.

The excretory bladder opens posteriorly on the dorsal side almost above the centre of the posterior sucker. There are three lymphatic canals on each side, two of which lie internal to the intestinal cæcum and the third one is external to it.

The two testes are oval, lobed and measure 0·3–0·32 × 0·135–0·16 mm. They are symmetrical, situated immediately behind the intestinal fork and are in contact with the intestinal cæca externally. The vasa efferentia were not seen clearly. The vesicula seminalis externa lies on the right side of the œsophageal bulb. The cirrus sac is almost pear-shaped and small and measures 0·094 × 0·0525 mm. Inside the cirrus sac is the tubular vesicula seminalis interna which is slightly curved. It is followed by a flask-shaped pars prostatica. The genital pore is situated centrally, slightly posterior to the bifurcation point from which the oral diverticula diverge towards the sides. It is surrounded by a genital sucker.

The ovary measuring 0·13 × 0·08 mm. is trilobed and is situated centrally, immediately behind the zone of the testes. The shell gland is situated centrally, immediately posterior to the ovary and slightly overlaps the anterior border of the posterior sucker. The Laurer's canal is present. The uterine coils fill almost all the inter-cæcal space excepting that occupied by the genital glands and pass anteriorly on the left side of the œsophageal bulb. The vitellaria are extra-cæcal and extend from the level of the

anterior border of the œsophageal bulb to a distance slightly short of the terminations of the intestinal cæca. Posteriorly a few follicles overlap the intestinal caeca. The ova are large but few and measure 0·074–0·082 × 0·042–0·044 mm.

The only trematode with which the form described here is related is *Helostomatis helostomatis* (MacCallum, 1905). It, however, differs from this species in the structure of the posterior sucker, the position of the testes with respect to the intestinal bifurcation, the relative position of the testes and the ovary and in some minor respects. These points of difference being very distinctive, it is considered necessary to create a new species for its reception for which the name *Helostomatis sakrei* is proposed.

Specific diagnosis of *Helostomatis sakrei* n. sp.—Body elliptical. Length 1·57 mm. Breadth 0·73 mm. Mouth ventral. Oral sucker 0·185 × 0·17 mm. Oral diverticula 0·215 mm. long. Posterior sucker with a hood anteriorly, 0·5 mm. dia. Œsophagus with bulb 0·3 mm. long. Intestinal cæca sinuous, terminating at the anterior border of the posterior sucker. Testes oval, lobed, symmetrical, measuring 0·3–0·32 × 0·135–0·16 mm. Cirrus sac measures 0·094 × 0·0525 mm. Genital pore slightly behind the diverticular fork. Ovary trilobed, central, immediately posterior to the testicular zone. Shell gland immediately behind ovary. Laurer's canal present. Lymphatic canals three on each side. Ova 0·074–0·082 × 0·042–0·044 mm.

Host.—*Labeo calbasu.*

Location.—Intestine.

Locality.—Poona (Bombay Presidency).

To accommodate the species described here it is found necessary to emend the diagnosis of *Helostomatis* Fukui, 1929 which has been raised to a generic status by Travassos (1934).

Emended diagnosis of *Helostomatis*.—Cladorchinæ: Body elliptical. Oral sucker with diverticula. Posterior sucker with a dorsal hood anteriorly. Œsophageal bulb present. Intestinal cæca extending posteriorly as far as the anterior border of posterior sucker. Genital pore pre-bifurcal. Genital sucker present. Cirrus sac present. Testes lobed, connubial, at about midbody. Laurer's canal present. Ovary entire or lobed, post-equatorial. Uterine coils inter-cæcal. Vitellaria lateral, for the most part extra-cæcal. Eggs few, large. Lymphatic canals three on each side.

Type-species.—*Helostomatis helostomatis* (MacCallum, 1905).

Neocladorchis n.g.

Neocladorchis poonaensis n.g., n. sp. (Fig. 12).

The material proposed to be described here was collected in 1933 by Professor J. N. Karve from the intestine and rectum of *Barbus dobsoni* in Poona.

The worms are subcylindrical and measure 1·8–5·85 × 0·6–1·4 mm. The broadest portion of the worm is immediately in front of the posterior sucker. The most anterior part of the body is sharply attenuated and appears to be somewhat distinct from the rest of the body. It is covered with small papillæ which in some cases are more prominent than others. The cuticle of the rest of the body is devoid of any armature.

The mouth is terminal and is surrounded by the oral sucker measuring 0·22–0·33 × 0·24–0·38 mm. This latter has two diverticula at its postero-lateral aspect, measuring 0·225–0·3 × 0·185–0·235 mm. These are oval and are together broader than the width of the oral sucker. The posterior sucker measures 0·63–1·07 mm. in diameter. The œsophagus is 0·36–0·68 mm. long and 0·125–0·175 mm. wide. Posteriorly it has a muscular bulb measuring 0·165–0·22 × 0·135–0·215. The œsophagus as well as the bulb are covered with glands. The intestinal cæca are somewhat sinuous and pass posteriorly as far as the anterior border of the posterior sucker. They may, in some cases, partially overlap the posterior sucker or might terminate slightly anterior to it.

The excretory bladder is oval and lies dorsal to the posterior sucker. The excretory pore is situated on the dorsal side slightly behind the middle of the posterior sucker. There are three lymphatic canals on each side of the body.

The testes are lobed and measure 0·36–0·825 × 0·28–0·67 mm. They are situated almost pre-equatorially and lie slightly oblique to each other. The horizontal zones of the testes may be touching or partially overlapping each other. The vesicula seminalis externa is much coiled and occasionally swollen at places. The cirrus sac is small, and measures 0·285–0·33 × 0·17–0·18 mm. The vesicula seminalis interna lying at the base of the cirrus sac is also coiled. The distal portion of the male genital duct, the pars prostatica, is a thin, straight duct. The cirrus sac opens into the genital atrium which communicates to the exterior by means of the genital pore. This latter is situated on the ventral side, in the region of the intestinal fork.

The ovary is round and measures 0·13–0·26 mm. in diameter. It is situated centrally, slightly in front of the posterior sucker. The shell gland is situated immediately posterior to the ovary, between the latter and the posterior sucker. The Laurer's canal is present. The uterus in the immature specimens is a zigzag tube passing centrally on the dorsal aspect. In the adult specimens the uterine coils fill up all the inter-cæcal area between the ovary and the posterior testis. Anteriorly the coils pass dorsally to the testes. The muscular terminal portion of the uterus opens into the genital atrium, posteriorly to the opening of the cirrus sac. The vitellaria are extra-cæcal and partially cæcal. They extend from some distance behind the posterior testis to the extremities of the intestinal cæca. The eggs measure 0·15–0·17 × 0·056–0·060 mm.

The form described here is closely related to the genera *Schizamphistomoides* Stunkard, 1925 and *Ophioxenos* Sumwalt, 1926, but it cannot be adequately fitted in any of these two genera. From the former it differs in having larger oral diverticula, in the testes being either touching or partially overlapping each other and the posterior extent of the intestinal cæca. From the latter it differs in respect of the size of the oral diverticula, the posterior extent of the intestinal cæca, the relative position of the two testes, the size of the vitelline follicles, the position of the ovary and the posterior extent of the uterus. For these reasons it is considered necessary to create a new genus for its reception for which the name *Neocladorchis* is proposed.

Diagnosis of *Neocladorchis* n.g.—Cladorchinæ : Body subcylindrical. Posterior sucker terminal. Oral sucker with fairly large diverticula. Intestinal cæca terminating close in front of posterior sucker or partially overlapping it. Œsophagus with a bulb. Genital pore central, bifurcal. Genital sucker absent. Testes oblique, lobed, for the most part pre-equatorial, touching or partially overlapping each other. Cirrus sac present. Ovary central, closely anterior to posterior sucker. Laurer's canal present. Uterus pre-ovarian. Vitellaria post-equatorial, lateral, extra-cæcal and cæcal, extending between posterior testis and posterior sucker. Lymphatic canals three on each side.

Type species.—*Neocladorchis poonaensis* Bhalerao, 1937.

Specific diagnosis of *Neocladorchis poonaensis* n. g., n. sp.—Anterior end covered with papillæ. Length 1·8–5·85 mm. Breadth 0·6–1·4 mm.

Oral sucker 0·22–0·33 × 0·24–0·38 mm. Oral diverticula 0·225–0·3 × 0·185–0·235 mm. Posterior sucker 0·63–1·07 mm. in diameter. Œsophagus 0·36–0·68 mm. long. Testes 0·36–0·825 × 0·28–0·67 mm. Cirrus sac 0·285–0·33 × 0·17–0·18 mm. Ovary 0·13–0·26 mm. in dia. Ova 0·15–0·17 × 0·056–0·060 mm.

Host.—*Barbus dobsoni.*

Location.—Intestine.

Locality.—Poona (Bombay Presidency).

All the type and cotype material of the species dealt with in this communication is to be deposited in the Helminthological Collection of the Imperial Veterinary Research Institute, Muktesar.

Summary.

The species *Bucephalopsis karvei* n. sp. has been described in detail and separated from the allied species. A survey of the work on Bucephalidæ has been given and the species *B. magnum*, *B. confusus* and *B. minimus* have been considered to be synonyms of *B. garui*. A key to the existing valid species of *Bucephalopsis* has been given.

The genus *Catoptroides* has been merged into the synonymy of *Phyllodistomum* and arguments for so doing have been advanced. Revised diagnosis of *Phyllodistomum* has been given.

Phyllodistomum shandrai n. sp. has been described and separated from the allied species. *P. sp.* has been briefly described. Key to the species of *Phyllodistomum* has been given.

Paramphistomum maplestoni n. sp. and *P. cuonum* n. sp., have been described and separated from the allied species *P. orthocœlium*. A few observations on *P. sp.* from a wild cat and *Diplodiscus amphichrus* from *Rana tigrina* have been recorded. *Paramphistomum cervi*, *P. sp.*, *Cotylophoron orientalis* and *Gastrothylax crumenifer* have been recorded from neelgai, hog deer and sable antelope respectively.

Helostomatis sakrei n. sp. has been described and separated from *H. helostomatis*. A revised diagnosis of *Helostomatis* has been given.

Neocladorchis poonaensis n.g., n. sp. has been described. The new genus *Neocladorchis* has been defined and separated from the allied genera.

ACKNOWLEDGMENTS.

It is my most pleasant duty to record here my indebtedness to Professor J. N. Karve, Dr. P. A. Maplestone, Dr. V. T. Korke and to the Director, Central Research Institute, Kasauli for the most valuable material placed at my disposal.

I wish also to record here my gratitude to Professor R. T. Leiper, F.R.S., for kindly supervising my work.

Explanation of Abbreviations.

C.V.D.	Common vitelline duct.
D.E.	Ductus ejaculatorius.
D.P.	Dorsal process.
E.P.	Excretory pore.
G.A.	Genital atrium.
G.P.	Genital pore.
G.T.	Genital tongue.
I.	Intestine.
O.	Œsophagus.
P.	Pharynx.
P.C.	Prostatic cells.
P.P.	Pars prostatica.
S.G.	Shell gland.
U.	Uterus.
V.	Vitellaria.
V.D.	Vitelline duct.
V.S.	Vesicula seminalis.
Y.R.	Yolk reservoir.

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A Morphological Study of Bovine Schistosomes.

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In a memoir on human and animal schistosomiasis (1934c), I reported the presence of bovine schistosomes for the first time in the Belgian Congo (Elisabethville). I also expressed the opinion that the bovine schistosomiasis in Katanga was of Rhodesian origin. It came from that same region from which Veglia & Leroux in 1929 described a new schistosome of Bovidae and Ovidae under the name *Schistosoma mattheei*, a species which has since given rise to some controversy. The material I brought back from Elisabethville readily lent itself to a morphological study of the two African bovine schistosomes.

Moreover, in this same region, Haut Katanga, I found that of the human cases of urinary schistosomiasis, 3·5 per cent. showed a mixture of two thirds eggs of the *S. haematobium* type with one third eggs of the bovine type. These cases were identical with those described from Southern Rhodesia by Blackie (1932), who deduced from them the infection of man by *S. mattheei*.

In a recent publication (1936) I showed that Blackie's deduction required substantiation and gave my reasons for thinking that these cases from Rhodesia and the Belgian Congo involved rather a polymorphism of eggs than a mixed infection of *S. haematobium* and *S. mattheei*.

In these papers I avoided taking sides in the dispute over the validity of the species *S. mattheei*, all the more so since a superficial observation of the bovine schistosomes from the Belgian Congo showed at once that some specimens agreed perfectly with the description of *S. mattheei*, while others at all points fitted the classical description of *S. bovis*. Professor Leiper very kindly allowed me to compare, at the London School of Hygiene and Tropical Medicine, some specimens from my collection with *S. mattheei* from Rhodesia and *S. bovis* from the Sudan.

This seems a propitious moment to undertake a morphological study of the bovine schistosomes of the Belgian Congo, now that Clayton Lane has just published in the Tropical Diseases Bulletin (1936) a complete review of the problem.

Present Position of the Problem.

Veglia & Leroux (1929) established the new species *S. mattheei* on the fact that the infection was exclusively intestinal (and not both intestinal and urinogenital as *S. bovis* was, according to Khalil's 1924 description), on the size of the adult worms and eggs, and on the fact that the intestinal caecum occupied half the length of the body instead of one third as in *S. bovis*.

Discussing these criteria in 1933, MacHattie, Mills & Chadwick (1933) rightly pointed out that Sonsino did not even mention the presence of *S. bovis* in the urinogenital tract. It was Panceri who put forward the theory of this location, on analogy with the human disease, and Khalil (1924) quoted this theory as an established fact by an error of interpretation.

Further, MacHattie & Chadwick (1932) in studying material from Irak were forced to the conclusion that "*Schistosoma mattheei* as described by Veglia & Leroux (1929) is indistinguishable from *Schistosoma bovis* as the latter occurs in Irak." These authors finally propose a new definition of *S. bovis*, considerably modifying that of Khalil (1924) and embodying the fundamental characters of the species created by Veglia & Leroux. Thus, the species *S. mattheei* would fall into the synonymy of *S. bovis*, defined as follows :

(a) Length, 12 to 28 mm.

(b) Measurements of the egg *in utero*, 90 to 205μ by 38 to 62μ. Measurements of the egg in the gut wall, 130 to 260μ by 40 to 95μ.

(c) Only rare specimens show the vitellaria confined to the posterior fourth of the female. In the vast majority of females the vitellaria occupy a little less than half the length of the body.

(d) The shape of the egg is usually that described by Khalil but approximately 0·1 per cent. of females show somewhat oval-shaped eggs.

(e) About 1 per cent. of females show many typically shaped *Schistosoma bovis* eggs *in utero* and in the same uterus one or more typically shaped *Schistosoma haematobium* eggs having the same measurements as the egg of this parasite of the human subject.

(f) Approximately 0·2 per cent. of females contain solely eggs of *Schistosoma haematobium* shape and measurement. Such females are only distinguishable from this parasite of man in that the vitellaria occupy approximately one half of the female length.

The Bovine Schistosomes from Katanga.

Paired schistosomes and some isolated females and males are found throughout the length of the alimentary canal, almost always in the mesenteric arcades of the 2nd order, rarely in veins of smaller calibre. Of 48 animals slaughtered at Elisabethville, 19 were parasitized. Infections were for the most part light : the minimum number of pairs recovered from any one animal was 6 and the maximum 44. The present study concerns only specimens found alive, immersed in physiological saline at room temperature until the moment of death, and then fixed in 10 per cent. glycerine in 70 per cent. alcohol. After some days the alcohol had evaporated leaving pure glycerine. Finally the worms were mounted in glycerine jelly.

I have already pointed out that by microscopic examination I could recognize schistosomes of the two types *S. mattheei* and *S. bovis*. The morphological differences which I noted between these two types are the more reliable in that all the specimens were collected and fixed in the same way and often came from the same animal. Four points claimed my attention : the size of the adult worms, the outline of the ovary, the size and shape of the eggs, and the length of the caecum in relation to the total length.

(a) The greater breadth of schistosomes of the *S. mattheei* type as compared with the *S. bovis* type is more characteristic than their greater length. Thus, whilst the breadth of a *Schistosoma bovis* does not reach twice the breadth of a uterine egg, that of a schistosome of the *S. mattheei* type exceeds four to five times the breadth of one of its uterine eggs. The microphotographs which appeared in a previous paper (1934-6) illustrate this point very clearly.

(b) In Schistosomes of the *S. mattheei* type the ovary has a fairly regular cylindrical shape with rounded ends, whilst in the *S. bovis* type the ovary is slightly twisted into a spiral (see Plate I, figures 1 & 2).

(c) The size of the eggs is extremely variable as between one worm and the next but remarkably constant for all the eggs from a single specimen, apart from the youngest egg which is distorted by fixation and is always smaller. So far as shape is concerned, I have found among my various specimens three well defined types which may be described as follows :

S. haematobium type—oval, lateral walls regularly convex. (Plate I, fig. 5.)

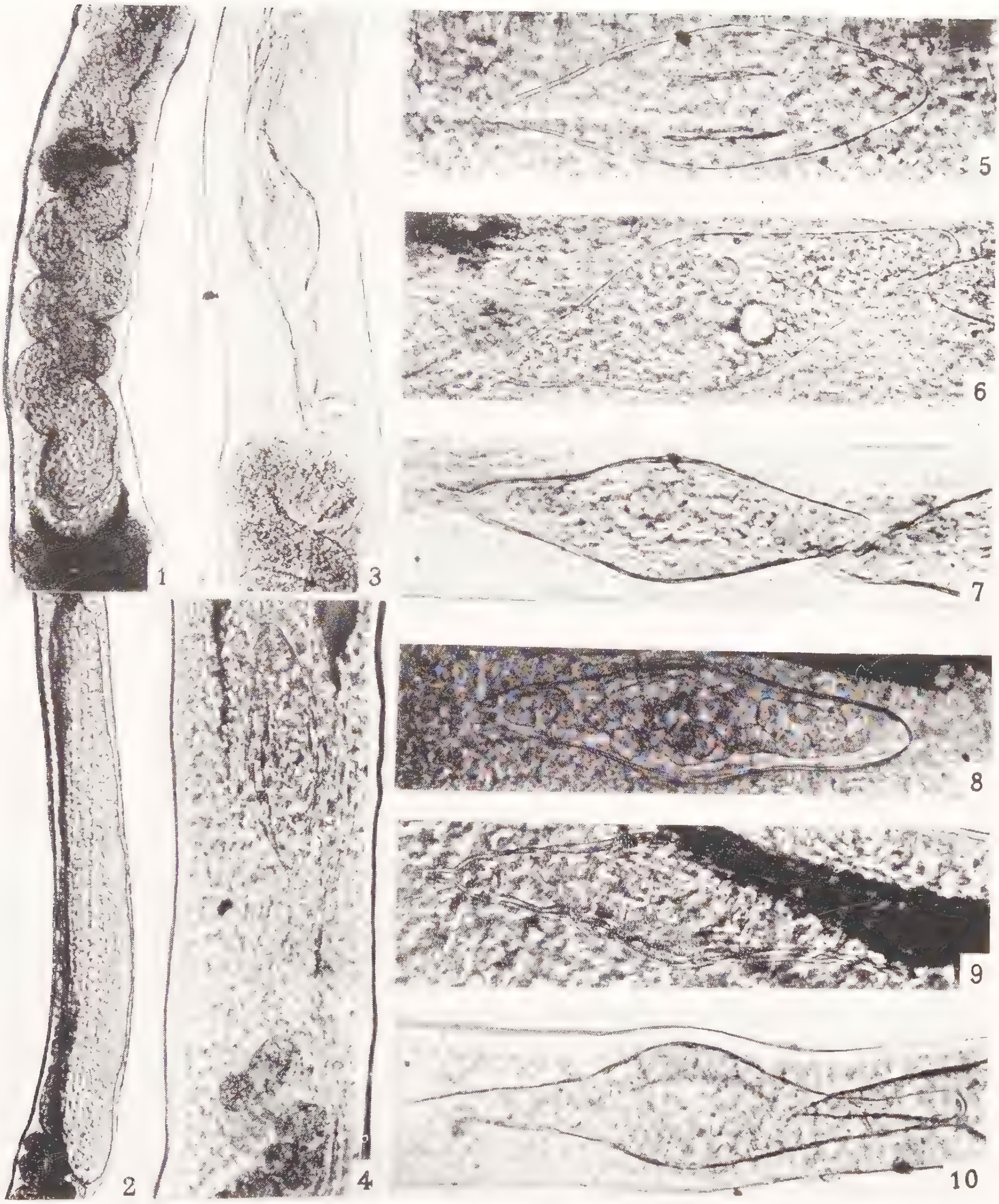
S. matthcei type—lozenge-shaped, lateral walls almost straight anteriorly and slightly concave posteriorly ; posterior extremity attenuated. (Plate I, fig. 6.)

S. bovis type—fusiform, lateral walls concave anteriorly and posteriorly, strongly convex centrally ; anterior and posterior extremities attenuated. (Plate I, fig. 10.)

The shape of the eggs from a single worm seems as constant as their size. Even the last egg formed, that nearest the ovary, conforms very sharply to one type or another (see Plate I, fig. 3, *S. bovis* type and fig. 4, *S. mattheei* type). This observation scarcely agrees with that of MacHattie & Chadwick according to which 1 per cent. of females show in the one uterus eggs of the *S. bovis* type and eggs of the *S. haematobium* type. These authors invoke a disease of the shell gland in order to explain these anomalies. Although this interpretation seems to us somewhat arbitrary, we must admit that in our material all the specimens were normal.

On the other hand, taking the shape and the size of the eggs into account together, there is considerable variation from one specimen to the other. Apart from the three types described above and figured in Plate I, figures 5, 6 and 10, there are innumerable shapes intermediate between the *S. mattheei* and *S. bovis* types, with the lateral walls less and less straight and the extremities more and more attenuated (Plate I, figs. 7, 8 & 9). Neither the shape of the eggs nor their size can be invoked as a constant and absolute criterion.

(d) The length of the caecum in relation to the length of the worm is also very variable. In their first note " On the morphology of a schistosome (*Schistosoma mattheei*, sp. nov.) from the sheep in the Cape Province " (1929) Veglia & Leroux give a table of comparative measurements of their species and of various descriptions of *S. bovis*. From this it is seen that, although Khalil (1924) gives the caecal length as one third or even one quarter of the total length, Montgomery (1906) and Bertolini (1908) assess the ratio as barely one half. Veglia & Leroux have promptly concluded that the species described by Montgomery and Bertolini were in fact *S. mattheei* and not *S. bovis*. In point of fact, these authors have too closely followed the data given by Khalil who doubtless had but



1. Ovary of *S. bovis*. 2. Ovary of *S. bovis* var. *mattheei*. 3. Youngest uterine egg of *S. bovis*. 4. Youngest uterine egg of *S. bovis* var. *mattheei*. 5. *S. haematobium*-like egg. 6. Shape like typical *S. mattheei*. 7, 8, 9. Shape intermediate between *S. mattheei* and *S. bovis*. 10. Typical *S. bovis*.

limited material in the Sudan. Bovine schistosomiasis does not actually exist in Egypt ; all the cases met with there come from the Sudan. I was myself able to confirm that specimens from the Sudan in the helminthological collection of the London School of Hygiene and Tropical Medicine were in agreement with Khalil's description. They had short caeca, similar to those of *S. haematobium*, and the eggs were all of the pure *S. bovis* type.

The results of measurements of one hundred females containing eggs of the *S. bovis* type and one hundred containing those of the pure *S. mattheei* type confirm what MacHattie & Chadwick state in their re-description of the species *S. bovis* : it is unusual to find a caecum whose length is less than half the total length of the worm. The graph (Fig 1.) shows very plainly that the curves for the *S. bovis* and *S. mattheei* females are very close to each other, and that the mean established by Khalil for *S. bovis* from the Sudan falls well below them.

Critical Analysis.

The essential criteria upon which the new species *Schistosoma mattheei* was based are variable from one specimen to another. There is a sharp demarcation neither in the shape and size of the eggs nor in the relative length of the caecum. The only differences which I have been able to observe are the width of the body, twice as great in *S. mattheei* as in *S. bovis*, and the outlines of the ovary, which have shown themselves characteristic of the two types in my material. It seems to me difficult to take one's stand on these differences in order to justify *S. mattheei* as a new species.

For some time there appear to have been in helminthology two trends of opinion, one tending to establish new species on ecological or biological characters, the other faithful to zoological tradition in admitting only morphological characters for differentiation. The two trends are especially marked among the schistosomes (*bovis-mattheei* and *haematobium-intercalatum*) and in *Onchocerca* (*volvulus-caecutiens*). From the purely systematic aspect it would be reasonable only to establish a new species when at least two new characters are available, both absolutely constant and mutually quite distinct one from the other. A difference in the shape of the eggs and the structure of the ovary or shell-gland, for example, would not constitute two distinct characters, since one might result from

the other. The error of creating an invalid species scarcely meets with as much consideration as the worthiness of showing it to be false. On the other hand it should be recognized that the description of such new species often entails the advantageous publication of more accurate data which would otherwise be lost, and the closer definition of the limits of variation which, for lack of material, are nearly always too narrow in the original descriptions of species.

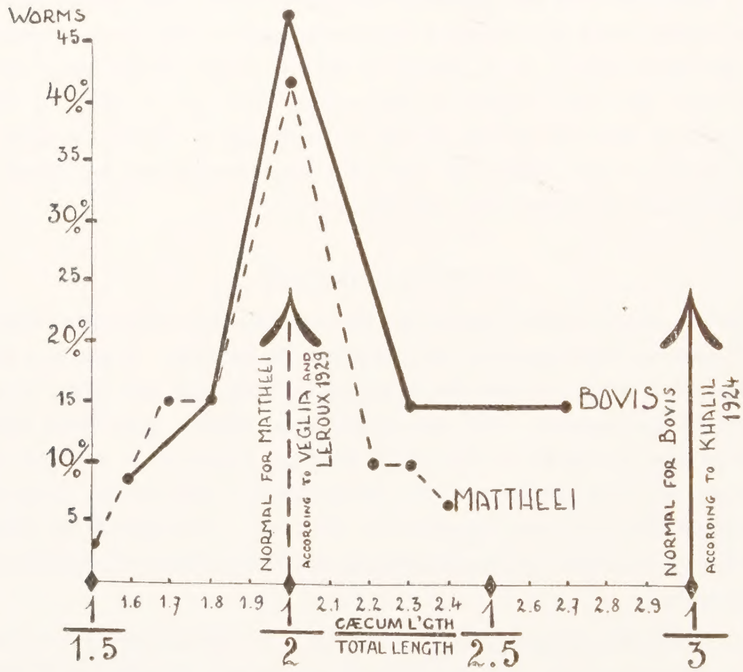


Fig. 1.—Length of caecum per 100 females of the *S. bovis* type and per 100 females of the *S. mattheei* type.

Referring once more to an earlier work in which we already remarked that, morphologically, the lines dividing *S. haematobium*, *S. bovis* and *S. intercalatum* (A. C. Fisher, 1934) are far from clear, we can here only insist that this applies still more to *S. mattheei*. It is this species which might justly have been called " *intercalatum* " in so far as the shape and size of the eggs are intermediate between those of *S. haematobium* and those

of *S. bovis,* as the accompanying microphotographs clearly show. Describing the new species *Schistosoma intercalatum,* associated with human intestinal schistosomiasis at Stanleyville (Belgian Congo), Fisher (1934) has represented the egg sizes of *S. haematobium, S. intercalatum* and *S. bovis* in the form of a graph. If to this the sizes of *S. mattheei* eggs are added, one obtains two intercalated curves which singularly approximate to the two extreme species, *S. haematobium* and *S. bovis.* Thus we have four species of schistosomes in which the males cannot be distinguished, and in which the principal female characters, caecal length and egg shape, appear to be far from constant. I have previously shown (1936) that *S. haematobium* can produce eggs in every way like those of *S. mattheei* and even those of *S. bovis.* On the other hand, this paper confirms the observations of MacHattie, Mills & Chadwick (1933) according to which the bovine schistosomes can produce eggs of the *S. haematobium* type (see Plate I, fig. 5). The one thing which clearly differentiates these species is their location in the host and the specificity of that host. Thus one might well consider the four species of schistosomes whose eggs have a terminal spine, *S. haematobium, S. intercalatum, S. mattheei* and *S. bovis,* as biological races of one and the same species. Since the conception of biological races is rarely employed in helminthology, although in current use in other branches of natural science, it seems reasonable to us to retain, along with the extreme species *S. haematobium* and *S. bovis,* the name *intercalatum* with the status of a variety of *S. haematobium* and the name *mattheei* with that of a variety of *S. bovis.*

Conclusions.

1. Following a morphological study of the bovine schistosomes collected in the Haut Katanga (Belgian Congo), it is proposed to relegate the species *Schistosoma mattheei* to the rank of a variety of *Schistosoma bovis.*

2. In view of the morphological relationships existing among the four schistosomes : *S. haematobium, S. intercalatum, S. mattheei* and *S. bovis,* we are led to the conclusion that these constitute biological races of a single species. However, we propose to conserve, as such, the most divergent species ; *Schistosoma haematobium* and *Schistosoma bovis,* and to refer the two intermediate species to *Schistosoma haematobium* var. *intercalatum* and *Schistosoma bovis* var. *mattheei.*

These two varieties may be defined as follows : *S. haematobium* var. *intercalatum* : Conforming in all respects to the classical description of *S. haematobium* but with eggs larger and of a lozenge shape resembling those of *S. bovis* var. *mattheei*. Location purely intestinal. Parasites of man apparently limited to the region of Stanleyville, on the banks of the Congo.

S. bovis var. *mattheei* : Conforming in all respects to the description of *S. bovis* as modified by MacHattie & Chadwick (1932). The eggs are very polymorphous, most frequently lozenge-shaped. For equal widths of eggs, the body is twice as wide as that of *S. bovis*. The ovary has a regular cylindrical shape.

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